REPORT

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THE CHOLERA-BACILLUS.

BY

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Research Scholar of the British Medical Association.

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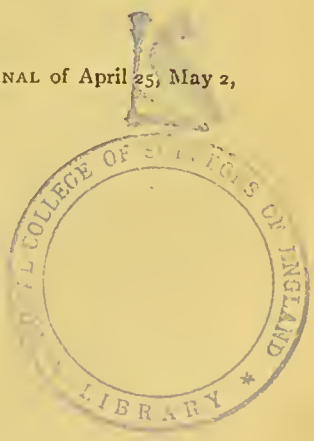

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In view of the great importance of the etiology of cholera, I have, for some months past, been engaged in observations on that subject; and, during the epidemic of Asiatic cholera in Paris, I went over there, and made some investigations on the bacteria present in the dejecta. The present paper gives the results of these investigations ; and to the report of my work, and the conclusions at which I have arrived, I have added an appendix, in which reference will be made to the observations of others, more especially to those of the English Commission.

In the study of every disease supposed to be caused by bacteria, three distinct lines of investigation must be followed. In the first place, the seat of the disease (blood, tissues, etc.) must be thoroughly investigated, with the view of discovering what micro-organisms are constantly present ; the characters of these micro-organisms must be studied, not only their microscopical characters, but also their behaviour on cultivation in various media ; and the bacteria must also be separated by cultivation from other morbid products. In the second place, it must be determined whether the bacteria that are constantly present are ever found in other diseases of the same species of animal, or under circumstances in which they probably ought, if they are the true cause, to have produced the disease, and have not done so. And, in the third place, it must be ascertained, by experiment on suitable animals, whether the organism so studied can reproduce the original disease.

In two very important points, cholera presents differences from most of the diseases which have as yet been investigated, and found to be dependent for their origin on certain bacteria. In the first place, in most of the diseases referred to, the cause is situated in the blood or tissues, and we have only one particular form of bacterium present, which is easily isolated and studied. In the case of cholera, the cause is apparently situated in the intestinal canal ; and, if there is any blood-affection at all, and there seems great reason to believe that there is, it is probably due to the presence of a chemical poison pro-

duced by this cause. Now, in the intestinal canal, there are normally large numbers of bacteria of different kinds ; and one must, therefore, find great difficulty in the first instance in picking out the organism specially associated with the disease. In the second place, cholera apparently only attacks man ; and, therefore, it is almost impossible to verify, by actual experiment, whether the particular organism fixed upon is, or is not, causally connected with the malady. However, we now know such a large number of bacteric diseases, that, even without this last proof, we are warranted by analogy in coming to a conclusion as to the causal or non-causal connection between cholera and any particular organism. In every case, where a definite form of organism has been discovered, in large numbers, in the diseased parts of an animal, and where this organism has never been found associated with other diseases, or normally present in the same animal, it has been found by experiment to be the cause of the disease. Fortunately, in the case of cholera, it seems as if, under certain circumstances, a somewhat similar affection can be induced in certain animals, and thus the foregoing difficulty is much diminished.

Now, the following seems to me to be the line of investigation to follow in the case of cholera, from the bacterial point of view ; it is, in fact, practically the line which was followed by Dr. Koch. In the first place, the blood and tissues would be carefully searched for bacteria, but attention would be more especially paid to the contents of the intestine. In every case examined, all the different forms of micro-organisms present in the evacuations would be carefully separated and studied. This would be done in a large number of cases, and then those forms only occasionally present would be rejected, and attention directed to those constantly present. If there were more than one of these, an important point would be to note which were generally present in greatest numbers. Their relation to the tissue of the intestinal wall would also be noted ; but this is of only secondary importance. The next point would be to determine which of these were only found in cholera, and which were found in other affections, as simple diarrhœa, dysentery, etc., or under circumstances in which Asiatic cholera was out of the question. Then the effects of these organisms on animals must be studied, and any facts observed which relate to their causal or non-causal connection with cholera. That there are numerous other points to be investigated is, of course, evident to all, but the foregoing was what I proposed to do in the first instance, if ever I had a suitable opportunity. I am aware that, owing to want of this opportunity, the following research falls far short of the ideal ; nevertheless, the facts narrated are important, taken in connection with the researches of others, and they are sufficient to enable me to discuss this interesting topic from personal knowledge.

Before going on to the consideration of these various points, it is important, and I think it may interest the members of the Association, to point out here how one distinguishes different bacteria from one another, as the method of distinction is not sufficiently appreciated in this country. By the mere form, it is quite impossible to distinguish any one variety of bacterium from others of which the form is somewhat similar. Thus the micrococci are all round bodies, and, except for slight differences in size or grouping—often quite inappreciable—they closely resemble each other ; and it would, therefore, be quite impossible to tell one species or variety from another, if the form alone were taken into account. And the same holds good with the bacilli and the spirilla. Indeed, taking into account the great variations in size and form shown by the same organism when grown in different media, it would be rash, and would certainly lead to error, if one attempted to determine the kind in any given case by microscopic appearance alone.

Further information may be obtained in some cases by the chemical reaction of the organism, but this is only applicable as the sole means of distinction in the case of the bacilli of tubercle and leprosy.[1] Thus the bacilli of tubercle and leprosy are distinguishable from all other known bacilli by the fact that, when they are stained in the first instance in a solution of fuchsin in anilin-water, and afterwards washed with dilute nitric acid, and then immersed in a contrast colour, for example, methylen-blue, they retain the fuchsin, while other forms of bacteria treated in a similar manner lose the fuchsin, and generally take up the contrast-stain. One can therefore say that, if red stained bacilli be present in a specimen treated in this manner, one has to do with the bacilli either of tubercle or of leprosy. These organisms can be distinguished from each other by a further chemical test; namely, if a mixture of tubercle-bacilli and ordinary bacteria be stained in a watery solution of fuchsin, washed in water, alcohol, etc., the tubercle-bacilli lose the stain, while the leprosy-bacilli, treated in a similar manner, retain it. Hence, reverting to the case above supposed, we can ascertain whether the bacilli seen were those of tubercle or leprosy, by staining another specimen in a watery solution of fuchsin. In this instance, the distinction is made not by the form, but by the chemical reactions, of the organism.

For the determination of other forms of bacteria, recourse must be had to various points in their life-history, more especially their mode of growth, the temperature at which they grow, the medium in which they flourish best, etc. Without entering at length into the materials employed in the cultivation of bacteria, I may state at once, what is now an acknowledged fact, that the greatest amount of information is to be derived from cultivation in meat-infusion rendered solid by the addition of gelatine. This material was first introduced with the view of enabling one to carry on pure cultivations of various bacteria, on the principle that, in a solid medium, bacteria can only grow where they fall or are planted, and that thus the presence of accidental contamination could be readily recognised, and the growth which is being studied be transferred to a fresh medium before it has become mixed with the extraneous organisms. It was soon found, however, that various organisms growing on this solid material could be readily distinguished from each other, even by the naked eye, by the form assumed by their colonies, by their effects on the gelatine (liquefaction, etc.), and by other characteristics; and thus, perhaps, the most important use to which this material is now put, is the distinction of different forms of organisms from one another. For this purpose, the gelatine is employed in three different ways.

The first we may call test-tube cultivations. In this method, a certain quantity of the gelatinised material is introduced, while still liquid, into a number of sterilised test-tubes plugged with cotton-wool; and, after the material has been sterilised by repeated boiling, it is allowed to solidify with the test-tube in a vertical position, and kept for a few days to see if it be pure. A long fine platinum wire, stuck into the end of a glass-rod, is sterilised by heat, and dipped into the material containing the pure cultivation of the organism. The cotton-wool plug being removed, with various precautions to prevent the entrance of dust into the tube, the infected wire is plunged into the jelly down to the bottom of the tube. It is then rapidly withdrawn, the wool-plug is re-inserted, and the tube placed at a suitable temperature. By the appearance of the growth, both on the surface of the jelly and along the tract of the wire, and by its effects on the jelly, much information may be derived as to the species under observation. Thus, one organism may grow

[1] Apparently the bacillus of syphilis can also be distinguished by chemical reaction. See Lustgarten's paper in the *Lancet*, March, 1885.

both on the surface of the jelly and along the needle-tract; another, though microscopically similar, may grow along the tract of the wire, and not on the surface; a third may liquefy the gelatine, and so forth.

The second method we may term slide-cultivations. Here a number of ordinary 3 × 1 microscopic slides are sterilised by heat, placed on a series of glass trays, in a dish containing moist blotting-paper, and covered by a bell-jar. On these slides some liquefied jelly is poured, and allowed to solidify. The wire, charged as before, is then rapidly drawn over several parts of the surface of the jelly. Bacteria are thus sown at various points along the tracts, and, growing there, produce colonies, the appearance of which can be studied under the microscope with a low power.

The third method, which may be called glass plate cultivations, is that which is also used for the examination of water, and for the separation of different forms of bacteria from one another. In this method, a minute quantity of the material containing bacteria is introduced into a tube of jelly, rendered liquid by keeping it for a few minutes at the body-temperature. The liquid jelly is then well shaken, so as to diffuse the bacteria throughout it, and is poured out on sterilised glass plates kept in a dish, arranged as for the last method. The jelly solidifies, and the bacteria, having been caught at various parts, grow there and form colonies, which may be readily recognised under the microscope with a low power. This mode of growth in colonies on glass plates is one of the best means of distinguishing different species of bacteria from one another. The exact form and size of the colonies depends to a considerable extent on the amount of gelatine used, although the general type remains the same. Hence 10 per cent of gelatine is now always employed, in order to have uniform results.

Further information as to difference in kind may in many cases be obtained by growing the organisms on the cut surface of cooked potatoes, in milk, in meat-infusion, etc. And then, again, by inoculation of animals, different results will be obtained with different bacteria.

Hence, in coming to a conclusion as to the nature of any given bacterium, its various characteristics, its form, its mode of growth, and its effects on animals, must be taken into consideration. Reliance on mere form alone, or, indeed, on any single characteristic, is not in any case satisfactory, and will in all probability lead to error. In the case of Koch's cholera-bacillus this is especially important, as a number of bacilli are now known which resemble it very closely in microscopic appearances. Hence, when the cholera-bacillus is spoken of, an organism is meant which, along with certain morphological characters, presents also certain peculiarities on cultivation on various materials.

I may now mention the cases of Asiatic cholera which I had the opportunity of examining in Paris, in November of last year. That they are very few in number, is due chiefly to the fact that the epidemic subsided very suddenly, and, though I timed my departure so as to be there at the height of the epidemic, I arrived quite at the end; but partly to the difficulty which I experienced in getting access to the cases in the hospitals. My best thanks are due to Dr. Paul Haensell, director of the laboratory at the Quinze-vingt Ophthalmic Hospital, and to the authorities of that hospital, for kindly placing the laboratory at my complete disposal.

In five cases I obtained dejecta from living patients, all males. The dejecta were received into clean bottles, and immediately taken to the laboratory, where a number of glass-plate cultivations were at once prepared as well as cover-glasses for microscopic examination.

1. Case ill 48 hours.
2. Case ill 24 hours.
3. Case ill 4 days, improving.
4. Case ill about 2 days, moribund.
5. Case ill 3 days, expected to recover.

In three instances I was able to obtain material from *post mortem* examinations. In each case portions of the intestine, more especially towards the lower end of the ileum, were ligatured in two places, and the enclosed pieces of gut taken to the laboratory in clean stoppered bottles, where they were opened, and cultivations made, and specimens prepared for microscopical examination. The portions of intestine were then put to harden in absolute alcohol.

6. Case ill about 2 days. *Post mortem* examination 12 hours after death.
7. Case ill about four days. *Post mortem* examination about 1½ hours after death.
8. Case ill about two days. *Post mortem* examination very shortly after death (within an hour). From this case I took specimens of the blood and internal organs as well.

In a ninth case, I obtained dejecta from a living patient, who had just been brought into the cholera-wards, supposed to be suffering from cholera; but when he was seen by the physician, it was pronounced not to be a case of cholera at all, and he was removed from the cholera-wards.

Results of the examination of these cases. On account of the small number of the cases at my disposal, I devoted my attention chiefly to Koch's cholera-bacilli; indeed, as far as I saw, they were the only organisms new to me which were constantly present. I kept and brought back cultivations of the other organisms which I found, and which were various straight bacilli, apparently of four kinds, and a few micrococci; but they seemed to be the same as those ordinarily found in the intestine.

Of the methods of examination before referred to, cultivation in nutrient jelly is the only one which yields satisfactory results in ascertaining what kinds of organisms are present, and in what proportionate numbers. Of the three methods before described, the glass-plate cultivations are the only ones which can be usefully employed in the first instance. The principle here is that the bacteria, being diffused through the fluid jelly, are separated from each other, and, being caught at various points when solidification occurs, each bacterium grows into a colony, which ultimately becomes visible to the naked eye, or with a low power of the microscope. If the experiment be successfully and properly performed, each colony will therefore represent a single bacterium; and, by counting the number of different colonies, one can arrive at an estimate of the relative numbers of different bacteria present in the original material.[2] In order to get a satisfactory result, the number of bacteria on each plate must not be great, otherwise there will be too many colonies, which will rapidly coalesce one with the other. The difficulty with dejecta is that, on account of the enormous numbers of bacteria present, it is difficult to introduce a small enough quantity into a tube. In order to effect this, I prepared a number of small sterilised flasks, plugged with cotton-wool, and containing distilled water, sterilised by repeated boiling.

[2] It is of course evident that there is room for fallacy in estimation by cultivation as well as in estimation by the microscope, because there may be bacteria present in the dejecta which will not grow in the ordinary nutrient jelly. Nevertheless, this fallacy is not nearly so serious as the fallacies of microscopical observation alone, because by far the greater majority of bacteria will grow readily in the nutrient jelly at a temperature of 20° C. And farther, as the cholera-bacilli grow very readily, one gets a much better idea of the myriads of these organisms which are present in cholera-dejecta, than one does by the use of the microscope alone.

A minute quantity of the dejecta was then introduced into this by means of a heated platinum-wire, the cotton-wool plug replaced, and the whole thoroughly shaken so as to diffuse the bacteria through it. Then, by means of a sterilised pipette, a drop was taken and put into a tube of liquefied jelly, and this was repeated in a number of tubes. These tubes were then thoroughly shaken up, their contents poured out on glass plates, as before described, and allowed to solidify. These vessels were afterwards kept at a temperature of about 20° C. (68° F.) In this manner a limited number of bacteria were obtained on each plate, and a careful study could be made of them. Another method, which I also employed in all cases, but which is not so good, was to inoculate a tube of jelly directly with the dejecta, liquefy it, shake it up, and pour one or two drops from the first tube into a second; shake up the second, pour a few drops from it into a third; repeat the process with a third, and a fourth, etc. Here the plates from the first two tubes were generally useless, on account of the large numbers of bacteria present, but the result in the third and other tubes was good.

The material employed was a meat-infusion containing 1 to 3 per cent. peptone, 10 per cent. gelatine, and one-tenth per cent. chloride of sodium, and rendered as nearly neutral as possible by carbonate of sodium. It is a very difficult matter exactly to neutralise this material; but Koch thinks that it is better to be slightly alkaline than slightly acid. I have not found that the exact reaction matters much, so long as the greater part of the acid is removed. My aim always is to get the material as nearly neutral as possible.

The temperature is also of considerable importance. It must not be too high, otherwise the gelatine will melt; nor too low, otherwise the cholera-bacilli will not grow. An average temperature of from 18° to 20° Cent. (64.4° to 68° Fahr.) is the best.

Along with the results obtained by cultivation, I will give the results of the microscopic examination of the material. A thin layer of the dejecta, or contents of the intestine, was spread on cover-glasses, and dried at the same time that the cultivations were made. These cover-glasses, if kept dry, remain good apparently for an indefinite length of time, and can be stained and examined at leisure. In Paris, the preparation and examination of the cultivations, and the separation of the different bacteria from one another, took up so much time that I was unable to examine these cover-glasses till some weeks later. The first estimation, and, as it turns out, the most reliable estimation, of the number and kinds of bacteria present, was made by cultivation from, and not by microscopical examination of, the dejecta.

No. 1. The dejecta contained a large amount of blood. On cultivation, the bacteria present were not numerous, as compared with the other cases. About 60 per cent. of the colonies which developed were colonies of Koch's comma-bacilli. On microscopic examination, large numbers of blood-corpuscles were seen, and only a few bacteria, among which only very few were well marked comma-bacilli.

No. 2. On cultivation, about 95 per cent. of the colonies which developed were Koch's comma-bacilli, or, as we may hereafter call them shortly, cholera-bacilli. This was the case in every plate, there being very few colonies of other kinds of bacteria. The dejecta in this case, then, contained almost a pure cultivation of cholera-bacilli. The result, on microscopic examination, was, however, by no means so definite. Certainly not half the bacilli were readily recognisable as comma-bacilli; but there were numerous slender organisms present, many of which, on close examination, were seen to be slightly curved; but, without the culture-test, it would have been quite impossible to decide whether or not these were cholera-bacilli.

No. 3. On cultivation, the majority of the colonies consisted of other bacilli than cholera-bacilli. I estimated that not more than 20

per cent. were cholera-bacilli. On microscopic examination, only a few comma-shaped organisms and spirilla were seen.

No. 4. As in No. 1, the dejecta contained a large amount of blood, and the result was very much the same.

No. 5. On cultivation, about fifty per cent. of the colonies were cholera-bacilli. Microscopical examination showed a smaller proportion of definitely comma-shaped organisms.

No. 6. In this and the two following cases the contents of the lower part of the small intestine were examined, and in this case and in No. 8 the contents of about the middle part of the jejunum were also taken. On cultivation from both situations, large numbers of cholera-bacilli (between eighty and ninety per cent.) were found to be present. From the microscopic examination, the estimate of distinctly comma-shaped organisms would have been very much less, but there were numbers of small bacilli, many of them showing a slight curve, but many in the same groups in which the curve was hardly or not at all noticeable, though they resembled the small curved ones in size and general appearance. In fact, the picture was very much like Fig. 1,

Fig. 1.—Cholera-bacilli which have been growing for four hours in meat-infusion, kept at the temperature of the human body.

and I have now little doubt that these smaller organisms were only rapidly growing cholera-bacilli.

No. 7. Cultivation showed not more than thirty or forty per cent. of cholera-bacilli. The great majority of the other organisms present were micrococci and not bacilli, as was usual in the other cases.

No. 8. The results of cultivation and microscopical examination of the contents of the intestine were almost identical with that of No. 6.

In the ninth case I failed entirely in finding any cholera-bacilli.

It will be evident, from a comparison of the results obtained in the foregoing cases by cultivation and by the microscope, that what I have before been saying as to the difficulty of distinguishing bacteria by the microscope alone is confirmed in this instance. Indeed, in the case of the cholera-bacilli, the difficulty is especially great. For, on the one hand, all comma-shaped organisms are not cholera-bacilli; we now know several curved organisms, having a very similar microscopic appearance, which it would be impossible to pick out from cholera-bacilli in a mixture of bacteria: such are Finkler's comma-bacilli, Flügge's cheese-bacilli, and others, which will be presently referred to. On the other hand, the cholera-bacilli vary very much in size and curvature, and, when growing rapidly, are small and very slightly curved, or indeed quite straight. Hence, here as elsewhere, the cultivation-characters must be mainly depended on.

Sections of the walls of the intestine were also made and stained with various aniline dyes, but I have found very few comma-shaped bacilli in them. When I spoke at the debate on cholera at the Royal Medical and Chirurgical Society in March, I had failed to find any; but since that time I have made a number of attempts to stain them, and I have been able to see a few faintly stained in the tissue in Case No. 8, after immersion of the sections in a saturated watery solution of fuchsin for twenty-four hours. I would not lay stress on this point, for two reasons. In the first place, Dr. Koch found the bacilli in the tissues, especially in the neighbourhood of Peyer's patches, at the

lower part of the ileum. Now, after hardening the tissues for some months in alcohol, I have found it very difficult to pick out Peyer's patches. In the second place, it is always much more difficult to stain bacteria in tissues than to stain them in cover-glass preparations. For the tissue has to be passed through various reagents, with the view of removing the excess of stain, and with the intention of leaving only the nuclei and the bacteria coloured. And during these processes the stain is very apt to be washed out of the bacteria as well. Various species of bacteria differ very much in the way in which they hold the stain under these circumstances, so that methods that will stain, for instance, the anthrax-bacilli admirably, will act very imperfectly, or not at all, when applied to tissue containing typhoid bacilli. Now, apparently the cholera-bacilli belong to the class which are apt to lose their colour in the various reagents in which the sections must be immersed; and, in any case, they are not deeply stained, as I can testify from observation of Dr. Koch's specimens. In presence, then, of the fact that Dr. Koch and others have found these bacilli in the intestinal wall in many cases, I am not prepared to say that where I did not find them they were not present. It is to Dr. Koch that we owe most of our knowledge as to staining bacteria, and it is, of course, quite probable that he may succeed where others fail. In any case, the presence or absence of these organisms in the intestinal wall does not, I think, affect the question as to the causal relation between these organisms and cholera.

I shall now proceed to describe the morphological characters and the appearance of the cultivations of the cholera-bacilli. And first I may state that I took care to ascertain, on my return from Paris, that I was working with Dr. Koch's organism. For I sent to Dr. Koch several tubes containing cultivations of the organisms which I had obtained, with the request that he would examine them, and let me know whether they were cultivations of his organism, and whether they were pure cultivations. Dr. Koch kindly examined them very carefully, and answered both my questions in the affirmative. I was thus able to go on with my observations, with confidence that I was dealing with the proper organism.

The cholera-bacillus varies very much in form, according to the conditions under which it is growing; but the general type is that of a short rod somewhat curved. I cannot describe it better than has been done by Dr. Koch, who says that it is at most about two-thirds the length of the tubercle-bacillus, and curved, the curve being commonly about that of a comma (see Fig. 3). The degree of curvature, and the length and thickness of the organism, vary, however, very much, as I have just said, with the conditions under which it is grown. The forms which it assumes, when it grows rapidly, may be readily studied in the following manner. A number of slides, with small depressions or cells hollowed out in their centre, are placed in beakers plugged with cotton-wool, and sterilised in an iron box raised for three hours to the temperature of 300° Fahr. When they are cool, a little vaseline is brushed round the margin of the cell, and the slides are laid on a sterilised glass plate, and protected from dust by a glass shade. A similar number of cover-glasses are cleaned and sterilised by passing them several times through the flame of a Bunsen burner, and placed on a pure glass plate under another shade. By means of a purified syringe, to the end of which is attached a narrow bent glass tube, a minute quantity (about one-third of a minim) of a pure infusion of meat, neutralised, and containing 3 per cent. of peptone, is placed on the centre of each of these cover-glasses. Each drop is then inoculated from a pure cultivation of cholera-bacilli. (The purity of the cultivation is easily ascertained by making glass-plate cultivations in nutrient jelly at the same time, as formerly described; and this, of course, ought always to be done.) The cover-glasses are now seized

in purified forceps, inverted, and placed over the cell on the slides, the edge of the cover-glass being pressed down so as to diffuse the vaseline all round. Care must be taken not to use too much vaseline, otherwise it may run over the glass, and become mixed with the cultivating fluid. With a little practice, one can easily manage to have the infected drop hanging from the middle of the cover-glass without any admixture with the vaseline. These slides are now placed in an incubator, kept at the temperature of the human body. After the lapse, say of an hour, one is removed, the cover-glass lifted off, inverted, placed on the top of the incubator under a glass shade, and dried rapidly. When dry, it is passed through the gas-flame three times, to fix the layer to the glass, and to render the albuminoid materials insoluble in water, and it is then stained in a suitable solution (fuchsin, methyl-violet, etc.). After staining, it is washed in water, or, after the methyl-violet, in weak acetic acid, dried thoroughly, and mounted in Canada-balsam. By removing slides at intervals of half an hour to an hour, and preparing the cover-glasses in the manner described, a beautiful series of permanent preparations, illustrating the mode of growth, may be obtained and studied at leisure.[3] To get a regular series, showing the successive stages of growth, the same amount of fluid and the same number of bacilli must be placed on each cover-glass. This is readily managed by inoculating, in the first instance, the flask of meat-infusion with the bacilli, and then placing it in an incubator for two or three hours. The bacilli grow and diffuse themselves through the liquid. Before use, this liquid is well shaken, and by means of a finely graduated syringe,[4] the same quantity of fluid, and hence probably the same number of bacilli, is placed on each cover-glass.

In the early specimens taken, say in from one to three or four hours, the appearances obtained are shown in Fig. 1. The bacilli are, as a rule, much smaller than in cultivations in gelatine, and their curve often very slight, or it may be not at all evident. As a rule, however, even those which appear straight will show, when examined with a high magnifying power, a thickening in the middle, which projects more to one side than to the other; that is to say, while one side appears quite straight, the other will appear somewhat convex. In some individuals, however, even this slight thickening will not be manifest. At first sight, one might think that the cultivation had become impure, but this is not the case, for the following reasons. In the first place, I have always tested the material used, and found that it was pure. Then these appearances are most noticeable in the specimens which have been one to three or four hours in the incubator. In the later specimens, the curved forms are much more numerous and marked than in the earlier, which would not have been the case had an impurity existed from the beginning. I have also often applied this method to well characterised forms of bacteria, to spores, etc., and I have never found any evidence of impurity, unless in one or two cases where the specimens had been kept in the incubator for about two days, and then only very few accidental forms were present as compared with the numbers of the organism originally introduced. But what is the most important point, and what definitely excludes the idea of contamination, is that I base this description only on the observation of chains of bacilli in which some of the members showed the well marked curve, and others not; I do not base it on the examination of individual isolated bacilli alone. The

[3] I have applied this method, with great success, to the study of other bacteria, as, for example, to the study of the mode of spore-formation and sprouting spores of the bacillus alvei, as will be described in the August number of the *Microscopical Journal*.

[4] See description of syringe in Sir J. Lister's paper on "Bacterium Lactis," in *Pathological Transactions*, 1878.

existence of this, which we may call the straight stage of the cholera-bacillus, is of great importance to bear in mind, as it is probably the form which many of the bacilli assume in the intestinal contents in the early period of cholera, and as it would be impossible to recognise them as cholera-bacilli by the microscope alone, more especially when mixed with other bacteria. Hence, the discrepancy between the microscopic estimate and the estimate by cultivation, with regard to the numbers of these bacilli present in any given case, a discrepancy not remarked by me alone, but by several other observers. Indeed, from the very first, Dr. Koch found it impossible to distinguish by the microscope alone, in a mixture of bacteria, these bacilli from "other very similar forms of intestinal bacilli."

If now we examine the specimens which have been in the incubator for a longer time (eight to ten hours) we find fewer small bacilli and a large number of larger distinctly curved forms, more especially at the edge of the drop ; and in many instances these are in pairs, forming the S-shaped form described by Dr. Koch. And there may also be a few spirillar forms, but I have not seen many in that stage. After this time development becomes less marked, and apparently soon ceases ; whether from exhaustion of the nutriment or of the oxygen contained in the cell I cannot say, but I think most probably for the latter reason.

If a cultivation in the nutrient jelly be examined after two or three days' growth, at a temperature of 18° C. to 20° C., most of the forms will be seen to be markedly curved, though considerable variation exists, and some almost straight rods may often be found (see Fig. 2). From a very early stage of their growth in gelatine, they tend to group themselves together, to form little irregular zoogloea-masses—the highly refracting particles seen in the cultivations with a low power, and likened by Koch to bits of glass ; while in the fluid jelly (for they render the gelatine fluid), there are large numbers swimming about very actively. Now in these zoogloea-masses, the bacilli are, as a rule, very distinctly curved. In those free in the fluid, the degree of curvature varies very much, some, as I have said, being almost straight. In the fluid, there will also be found S-shaped forms, consisting apparently of two organisms united end to end with the curves in opposite directions. In some cases, the union occurs with the curves in

Fig. 2.—Cholera-bacilli from a cultivation in the nutrient jelly.

the same direction, as in the numeral 3. Longer spirillum-like forms may also be observed, likewise evidently composed of a row of comma-bacilli. If the cultivation be examined after five or six weeks, definite spirillar forms will be seen (how formed, whether from continued elongation and twisting of a single individual, or from fusion of the individual members of a chain, I cannot say). The spirals are uniform in thickness throughout, and do not show any trace of division (see Fig. 3).

Examined under a high power (one twenty-fifth oil-immersion lens), bacilli are very often seen, which do not stain equally throughout, but in which there may be two or three circular parts in which the stain is different in intensity from that in the rest of the rod. Examined on a dark ground, many of the rods are seen to be somewhat beaded, as is so often observed in other bacilli. It is not always possible to demonstrate this beaded appearance in every specimen of these bacilli, hence it probably depends

Fig. 3.—Cholera-bacilli from an old cultivation in the nutrient jelly, showing the spirillar forms.

partly on the amount of stain taken up, and partly on the stage of growth of the organism. Mr. E. M. Nelson also states that he has observed flagella in many specimens of this organism, generally one at each end (see Fig. 4). To my eye, these flagella appear very in-

Fig. 4.—A cholera-bacillus from a drawing by Mr. E. M. Nelson, showing the flagella. It can also be seen that the substance of the rod is not uniform, but may be resolved into three granules. (This and all the drawings of the bacilli have been made with a $\frac{1}{25}$ oil immersion-lens. In this case, a high eyepiece has been employed.) The flagella have come out far too thick in the woodcut.

definite; but my vision is not so sharp as Mr. Nelson's, and I am the more inclined to accept his observation, as more than one independent observer has drawn the flagella in specimens demonstrated by Mr. Nelson, without having been told what they were to see. That flagella should exist is in the highest degree probable.

The amount of curvature is, as far as I can judge, dependent to a great extent on the rapidity of growth of the organism. The more rapidly it grows, the shorter it is, and the less marked is the curvature. The more slowly it grows within certain limits, the more marked is the curvature, and the greater the number of S-shaped and spirillar forms. The most perfect specimen which I have seen, where the curve was marked in all the organisms, was obtained from a very slow and imperfect cultivation in jelly, which had not been neutralised, and which was distinctly acid. And I am told on good authority, though I have not yet had time to repeat the observation for myself, that, if 5 per cent. of alcohol be added to the nutrient jelly, the growth is slow, and the great majority of the forms observed are spirillar.

I do not wish, however, to attach too much importance to the mere rapidity of growth as modifying the morphological characters of this organism, because I think other as yet undefined conditions also play an important part. One of the most peculiar forms which I have seen was found in the contents of the large intestine of the guinea-pigs, which died after injection of cholera-bacilli. I tested the fluid by cultivation at the time very carefully, and found that it contained almost a pure cultivation of cholera-bacilli; there was certainly not more than one other kind of bacterium for every hundred cholera-bacilli. The appearance of this material, on microscopical examination, after staining, is shown in the accompanying figure (see Fig. 5). Large fat, coiled, almost worm-like organisms, will be seen, which, as I know by cultivation, are cholera-bacilli, but which could not be recognised as such by the microscope alone.

Dr. Klein has also described a peculiar appearance when these

organisms are grown on agar-agar jelly at the temperature of 18° Cent. to 20° Cent. According to him, the bacillus becomes vacuolated, and this clear space in the centre increases in size till the remains of the bacillus form a ring. He showed a beautiful specimen in support of this view at the recent debate at the Royal Medical and Chirurgical Society. I have not yet come across this appearance, but I am looking for it.

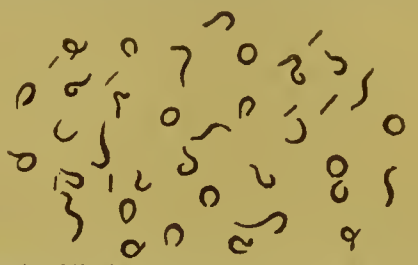

Fig. 5.—Contents of the large intestine of a guinea-pig, killed by the injection of cholera-bacilli into the duodenum. (The organisms are too thick in the woodcut.)

Leaving now the morphological characters which, though interesting, are the least important from a diagnostic point of view, I shall go on to describe the appearances on cultivation. I need not enter minutely into this matter, as I have nothing to add to Koch's description, which was published in the JOURNAL last year, and I shall therefore only enumerate the chief points.

In glass-plate cultivations, the colonies are evident in about twenty-four hours, and appear, under a low power, as small, somewhat irregular pale masses (see Fig. 6). These gradually increase in size, and, where

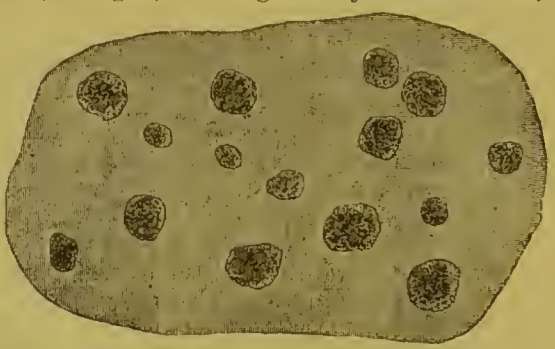

Fig. 6.—Colonies of cholera-bacilli in glass-plate cultivation, after 24 hours' growth. The larger colonies are at the surface of the gelatine, × 80.

near the surface of the jelly, a small depression forms over them, so that, on looking from the side at the surface of such a cultivation, it presents numerous little depressions instead of the original smooth surface of the gelatine, each depression corresponding to a colony of these bacteria. As the colony increases in size it becomes less compact, and the gelatine in the immediate vicinity becomes fluid. At this stage, the appearance is that of an irregular shaped mass of highly refracting granules, in the centre of a small area of fluid jelly, and floating about in the fluid are also other small refracting masses (see Fig. 7). When examined with a high power, these masses are seen to be aggregations (zoogloea-masses) of comma-shaped bacilli, and the fluid is seen to contain large numbers of very actively moving bacilli. The character of the movement is very difficult to determine on account of its great rapidity, but the S-shaped and spirillar forms move in a distinctly corkscrew manner. I do not think that the single bacilli swim in this spiral manner, but of this I am not quite certain. When a bacillus is about to become incorporated in a zoogloea-mass, its motion is very character-

istic. It moves backwards and forwards in part of the arc of a circle, the curvature being directed towards the centre of the circle, and away from the mass to which it is ultimately to be attached. After floating backwards and forwards in this way for some time—a movement which, I think, can only be accounted for by the presence of flagella—the range of movement becomes less and less, till the organism comes to rest at the edge of the mass.

Fig. 7.—Colony of cholera-bacilli in glass-plate cultivation, after three days' growth, × 120.

The colony goes on increasing in size for a few days, but ultimately ceases to extend or extends only very slowly. Dr. Koch reckons the ultimate extent of the colony at about one millimètre. This depends apparently on the amount of gelatine present, and is the result when ten per cent. is employed; but apparently, if five or three per cent. gelatine be used, the colony may attain a considerably larger size than that mentioned. Something, I think, also depends on the amount of peptone added to the cultivating material. These appearances on glass-plate cultivations, taken as a whole, are, so far as I am aware, peculiar to the cholera-bacilli. I know no other organism which forms colonies on plates, which cannot be distinguished from those of the cholera-bacilli by a low power of the microscope.

The test-tube cultivations are also characteristic, but hardly so markedly as the glass-plate cultivations. In twenty-four hours, at a temperature of 18° Cent., growth is evident along the needle-track as

Fig. 8.—Test-tube cultivation of cholera-bacilli after two days.

a whitish line, broader at the upper part and gradually tapering to the lower (the exact appearance depends of course on the size of the

platinum wire employed ; in all cases where a typical appearance is wanted, as thin a wire as possible should be used). At the upper part the gelatine begins to evaporate, and there is a slight depression. During the next twenty-four hours the growth becomes more marked, and the depression increases in size so as to look like an air-bubble at the top of the track (see Fig. 8). In the following days the jelly at the top becomes liquid, and this liquidity extends gradually to the bottom of the track ; thus there is a funnel-shaped appearance, from the greater amount of fluid at the top than at the bottom. At the same time, the mass of bacilli falls to the bottom of the fluid and assumes a somewhat rosy colour, so that there is a rose-coloured convoluted string running down the lower part of the track. The fluid at the upper part, which in about a week has extended to the sides of the test-tube, becomes clear, except a very thin layer at the top, which remains opalescent, the top itself being often covered with a very fine scum. Scattered over the solid gelatine forming the sides of the funnel are seen numerous small irregular highly refracting particles. These are the small zooglœa-masses which have fallen to the sides and bottom of the funnel-shaped cavity (see Fig. 9). The rapidity with which the gelatine becomes liquid depends very much on the amount of gelatine, and possibly of peptone, present, as before remarked. In about three weeks, the jelly in the tube becomes entirely liquid, and then we have a clear fluid with a somewhat rosy mass at the bottom, a fine scum at the surface, and a narrow layer of opacity beneath it. In such a tube, the bacilli will be found alive after six or seven weeks from the date of inoculation. No one of these characters is of itself peculiar to the cholera-bacilli. They must all be looked at together, and the rapidity of growth must be taken into consideration as well. To my mind, the most typical appearance is that of the highly refracting particles lying on the side of the funnel, the liquid in the neighbourhood being quite clear. The only other organism that I know which produces a somewhat similar appearance is Flügge's comma-bacilli, to be mentioned presently.

Fig. 9.—Test-tube cultivation of cholera-bacilli after ten days. A very large air-bubble is seen, as well as the other characters described.

I have previously mentioned that the reaction of the material should be neutral, or slightly alkaline ; and Dr. Koch did not think that this

bacillus would grow at all in jelly which had not been neutralised, that is, which was distinctly acid in reaction. Not that he holds that all acids would prevent growth ; for he points out that the surface of potatoes is acid, and yet these bacilli can be made to grow on them. I have, however, in two or three instances, obtained distinct growth of the cholera-bacilli in jelly which had not been neutralised, and which was found, when tested, to be distinctly acid. The growth was very slight ; and, in fourteen days, there was only a thin whitish growth along the track of the needle (not more than is formed by the typhoid-bacillus in neutral jelly in the same time), and there was no liquefaction of the jelly, nor did the growth spread over the surface of the jelly. The air-bubble appearance was also present. As I have previously mentioned, the comma-shape was well marked in all the bacilli in these cultivations.

In the neutralised meat-infusion with peptone, made solid by agar-agar, the cholera-bacillus grows fairly rapidly; but it does not produce any liquefaction of the material, nor is the growth distinguishable from that formed by many other kinds of bacteria. Indeed, it is a very curious fact that agar-agar material is of very little use, compared with gelatine, as a medium for distinguishing bacteria from one another, many forms growing almost in precisely the same way in it.

On the cut surface of boiled potatoes, the bacillus does not grow at a low temperature at all ; but, at the body-temperature, it grows fairly rapidly, and forms a brownish layer, which, according to Dr. Koch, closely resembles that formed by the bacillus of glanders.

It grows readily on blood-serum, at the temperature of the body, and liquefies it.

When grown in meat-jelly, containing 3 per cent. of peptone, a smell is evolved of a somewhat fæcal character, but not very strong. This smell is said by Nicati to resemble the characteristic odour of cholera-evacuations ; but with regard to this, I cannot speak from personal knowledge, for I did not pay particular attention to the odour of the cholera-evacuations. When grown in meat-jelly containing no pepton, or only one per cent., I have not detected any odour at all.

Dr. Koch has stated that these bacilli, when dried, rapidly lose their vitality; in three hours, they are completely dead. I have had no difficulty in confirming this observation.

A very important point is the behaviour of this organism in gastric juice—important with regard to the etiology of the disease, and also important in reference to conclusions drawn by Dr. Klein from the results which he has obtained on this matter, to which I shall refer in the appendix to this report. I feel it, therefore, necessary to go into the details of my experiments on this subject, instead of merely stating my results.

Dr Koch tells us two facts with regard to the relation of acids to the cholera-bacilli ; first, that certain acids, more especially the mineral acids, when present in the cultivating materials in small quantities, prevent the development of the bacilli ; and, secondly, that the bacilli are killed in the stomach. The latter statement is based on the fact that, after feeding animals with cultivations of comma-bacilli, and then killing them, and testing the contents of the stomach and intestines by cultivation, he failed to find any comma-bacilli. I have made a number of experiments on this subject ; and the method employed will, perhaps, be best understood if I describe an experiment which I performed, in presence of Sir Joseph Lister and some other gentlemen, with the view of making certain that there was no fallacy in the experiments, as they seemed to be opposed to experiments referred to by Dr. Klein. The explanation of the discrepancy between our statements will be given in the appendix ; in the meantime, I shall give my own experiments.

The normal proportion of hydrochloric acid in gastric juice is

generally given as 0.2 per cent.; and in the *Handbook for the Physiological Laboratory* the following mode of preparation is described by Dr. Lauder Brunton. "The ordinary strong hydrochloric acid, specific gravity 1.16, contains 31.8 per cent. by weight of hydrochloric acid gas. To prepare a dilute acid containing 0.2 per cent. of real hydrochloric acid, measure out, with a graduated pipette, 6.25 cubic centimètres of such acid into a litre flask; fill the flask up to the neck with distilled water, and shake so as to mix properly." I therefore prepared a 0.2 per cent. solution of hydrochloric acid in the above manner, and placed it in the incubator, as I thought, from previous experiments, that it acted more quickly at the temperature of the body than at the ordinary winter-temperature. On the previous day, I had inoculated with cholera-bacilli a flask containing neutralised meat-infusion and 1 per cent. pepton. This flask had been kept in the incubator for twenty-four hours; and the fluid was opalescent, from the presence of innumerable cholera-bacilli. I also had at hand a number of tubes containing the nutrient jelly, which had been rendered liquid at the temperature of the body; and also a number of covered dishes containing sterilised glass plates, on which to pour out the jelly. A control-experiment was first performed, to show that the addition of one or two drops of this dilute acid to the jelly did not interfere with the growth of the cholera-bacilli. To an ounce of distilled water were added two drachms of the turbid meat-infusion, containing cholera-bacilli; and the mixture was thoroughly shaken up. A tube of the liquefied jelly was now taken, and two minims of the dilute acid introduced, and then one minim of the diluted cultivation of cholera-bacilli. The material was then shaken up, poured out on a glass plate, and allowed to solidify. After it had solidified, the plate was transferred to a room kept at the temperature of 18° to 20° Cent. In thirty-six hours, enormous numbers of colonies of cholera-bacilli were seen throughout the gelatine; in other words, this amount of acid had not at all interfered with the growth of the bacilli. I may here say that I have performed at various times a number of similar control-experiments, with the same result. This control-experiment having been set a-going, I then took one ounce of the warm dilute acid, and added to it two drachms of the cultivation of cholera-bacilli. The mixture was then stirred; and, at various intervals of time, I added one drop of this mixture, with a pure pipette, to a tube of liquefied jelly. The jelly was then thoroughly shaken up, and poured out on a glass plate in a covered dish. After solidification (in about an hour), these dishes were placed at a temperature of from 18° to 20° Cent. I may give this particular experiment in a tabular form, with the result; and I would remind the reader of the result of the control-experiment in which the cultivation of cholera-bacilli had been diluted to the same extent, and in which, as a result, enormous numbers of colonies of cholera-bacilli developed.

No.	Length of Time the Acid Acted.	Result.
1	20 sec.	A large number of colonies, but not nearly so many as in control-experiment.
2	30 sec.	Number of colonies much fewer.
3	9 min., 56 sec	No development.
4	10 min., 16 sec.	,,
5	22 min., 30 sec.	,,
6	22 min., 45 sec.	,,

At the same time, I tested an artificial gastric juice, made by taking one ounce of the above dilute acid, and adding to it one grain of pepsin. After this had stood in the incubator for about ten minutes,

two drachms of the cultivation of cholera-bacilli were added to it, and tests were taken, as in the former experiment. A similar control-experiment to the above was also done, with the same result, that countless colonies of cholera-bacilli developed.

No.	Length of Time the Gastric Juice Acted.	Result.
1	45 sec.	A considerable number of colonies.
2	1 min., 15 sec.	A few colonies sparsely distributed over the plate.
3	9 min., 25 sec.	No development.
4	9 min., 35 sec.	,,
5	22 min., 45 sec.	,,
6	23 min.	,,

On the same occasion, also, I made a dilute acid solution containing only 0.02 hydrochloric acid, and tested its action in a similar manner. Up to fifteen minutes, but little difference was noticeable between the number of colonies on these plates and that in the control-experiment; but, in tests taken after half an hour, the colonies were not so numerous.

The meaning of the above experiments is to my mind quite clear; namely, that artificial gastric juice containing acid of the strength of 2 to 1250 (for the addition of two drachms of the cultivating fluid to the ounce of the gastric juice reduced its strength to the above), or acid of that strength alone at the body-temperature, very rapidly kills cholera-bacilli, so that in ten minutes none are left alive. As a matter of fact, I have ascertained that acid of this strength kills all the bacilli in two to three minutes. It might be thought that the acid added to the gelatine prevented their development, but the control-experiments dispose of that objection. Or it might be said that they have absorbed a certain amount of acid, and cannot get rid of it in the gelatine; whereas, if they were in a fluid, they would lose it again, and then grow. But they were put at once into fluid gelatine, and it was about an hour before the gelatine solidified; so that there was plenty of time for osmosis to occur. But I have disposed of this by the following experiment. A mixture of dilute acid and bacilli, prepared as above, was tested in the same manner, as follows.

No.	Length of Time the Acid Acted.	Result.
1	20 sec.	A considerable number of colonies.
2	65 sec.	Very few colonies.
3	2 min.	No development.
4	3 min.	,,
5	4.43 min.	,,

At 5.50 minutes, a drop was put into a flask containing about three ounces of an alkaline meat-infusion, with peptone. This was then placed in the incubator, at the temperature of the human body. No development occurred, although it was kept for several days.

It is worthy of remark that, had I not used the glass-plate method of cultivation, but the test-tube cultivations instead, I should have got quite an erroneous impression. Thus, in the above experiment, I also inoculated a number of test-tubes with the same mixture, as follows.

No.	Length of Time the Acid Acted.	Result.
1	45 sec.	No development.
2	1 min., 30 sec.	,,
3	2 min., 32 sec.	,,
4	3 min., 32 sec.	,,
5	5 min., 5 sec.	,,

Here the result is evidently due to the fact that, with the bacilli, acid was also carried in, and that it either continued to act, and destroyed the bacilli, as is, I think, most probable, or soaked into the gelatine around the needle-track, and prevented the development of the bacilli. It is in order to stop the action of the acid on the bacilli at once, that I always put the drop into liquefied gelatine.[1]

I may say that the above are not the only experiments I have performed to test this matter ; I have repeated them several times, and always with the same result. There is one other point, however, which the above experiments teach ; namely, that at certain stages of their existence these bacilli are more easily killed than at others, as shown by the fewer number of colonies in the successive plates, most probably due to difference in vitality in different bacilli. I am inclined to think that the younger bacilli are more easily killed than the older, because in one experiment, where I used a cultivation twelve hours old, and which was just beginning to become opalescent, I got no development, except in the control-experiments ; even thirty seconds' immersion in the acid had killed them. At what age these bacilli are most vigorous, I have not tried to ascertain ; but, if it were important, some idea might be obtained with a little trouble.

I have tried other strengths of acid, as, for example, 1 to 1850, in which I found, in one set of experiments, that the bacilli were destroyed in twenty-five minutes. (They may have been destroyed much sooner ; for I took the first three tests in four to five minutes, when plenty grew ; and the next three in twenty-four to twenty-five minutes, when none developed.) I have also tried 1 part of the commercial acid to 1000 parts of water ; that is, six times more dilute than the strength of acid in the gastric juice, as given by Dr. Brunton. With this, I got numerous colonies after twenty minutes.

The results obtained, then, so far, are that, in all the cases examined, Koch's cholera-bacillus was present, and generally in large numbers ; and that I found no other organism constantly present which was new to me. Further, in the one case (No. 8) in which I took specimens of the blood and portions of the internal organs, careful microscopic investigation failed entirely to show the presence of bacteria of any kind in the organs or blood. The observations as to the form, mode of growth, and general characteristics of the cholera-bacillus, also confirm Koch's statements in all essential particulars.

We now come to the second point ; namely, whether Koch's cholera-bacilli can be found in any other disease than in Asiatic cholera, or under circumstances in which any possible connection with Asiatic cholera is out of the question. In other words, is this organism peculiar to Asiatic cholera, or not ? The extreme importance of this question, in presence of the difficulty in getting decisive evidence one way or another from animals, will be evident to all, and I shall return to this point subsequently. In the meantime, it must be evident that, as it is the almost unanimous statement of various observers that this organism is always present in Asiatic cholera, it follows that, unless it be peculiar

[1] I have described these experiments in detail, also, because I think this is a very useful method of testing the action of antiseptics on other bacteria, and better for some purposes than Dr. Koch's method with the threads.

to this disease, it should be widely distributed throughout the world, and should, therefore, be readily found. Although my investigations on this matter are much more complete than on the first point, I need not take up much space with its discussion, as I have entirely failed to find this organism except in cases of Asiatic cholera. A few remarks on the materials examined will, therefore, be sufficient.

But, in the first place, I must point out that there are other comma-shaped bacilli than those found in cholera, which are, however, easily distinguishable from the latter. Of these, two liquefy gelatine, and must be more specially alluded to, namely, Finkler's and Deneke's, or Flügge's. Another has also been discovered by Miller in a carious tooth, which very closely resembles Finkler's, and is probably the same, so that I need not describe it specially. For the opportunity of studying these bacteria, I am indebted to Dr. Koch, who sent them to me.

Finkler's comma-bacillus was found by him in an epidemic of cholera nostras in Bonn, and was at first thought to be identical with the organism of Asiatic cholera ; but it has now been clearly shown to be a different organism. When examined without staining, it is seen to be larger than the cholera-bacillus, and the curvature is not so marked. The spirillar forms are also rarer, and the turns are not so numerous. It grows rapidly in gelatine ; the colonies are round, smooth, and it liquefies the gelatine extremely rapidly. In the test-tube cultivation (see Fig. 10), the gelatine becomes very rapidly fluid, so that, in

Fig. 10.—Test-tube cultivation of Finkler's comma-bacilli 48 hours.

twenty-four hours, it may reach the margin of the tube at the surface, and also extend down to the bottom of the needle-track. The fluid is also uniformly muddy. On potatoes, it grows rapidly at the temperature of 18° Cent., forming a thick greyish-yellow layer over the surface of the potato. It produces a strong fæcal or urinous odour.

Flügge's or Deneke's comma-shaped bacillus was found in a piece of old cheese. It resembles the cholera-bacillus more nearly than does Finkler's, but it is easily distinguishable from it. Seen in the unstained condition, it is smaller than the cholera-bacillus, and the spirillar forms are more frequent, but they are not so long as those in cholera, and also the individual bacilli forming the spirilla are attached rather at an angle than in a regular curve. It grows rapidly in the nutrient jelly, and its young colonies are very dark, round, and

of regular contour. They are irregularly granular. Liquefaction occurs much more rapidly than in the cholera-bacillus, but not so rapidly as in the case of Finkler's. A colony of Flügge's bacillus, twenty-four hours old, is very like a colony of the cholera-bacillus three or four days old. In test-tube cultivations, liquefaction occurs much more rapidly with Flügge's than with the cholera-bacillus; and here, again, a cultivation two days old is very like a cultivation of the cholera-bacillus a week old (see Fig. 11); but the fluid is somewhat

Fig. 11.—Test-tube cultivation of Flügge's comma-bacilli 48 hours old.

turbid, and the liquefaction at the lower part of the needle-track is more extensive than with the cholera-bacillus. It does not grow at all on potatoes.

I may perhaps conveniently tabulate the differences between these bacilli. (See table on next page.)

It will be evident that, taking all these characters together, it is easy to distinguish these organisms from one another. It is not so easy to describe their differences as to distinguish the variety in practice. When mixed together in the same glass-plate cultivation, one can pick out the three kinds with the greatest ease.

There are also other comma-shaped bacilli, but they do not liquefy gelatine. Indeed, several of them will not grow in the material used for cultivating the cholera-bacillus. Of these, I may mention three.

In Dr. Koch's report on cholera, he mentions that he found in water, in the neighbourhood of Calcutta, a comma-shaped bacillus, which, however, differed in important particulars from the bacillus of cholera. It may be best if I simply quote Dr. Koch's statement. "Only once did I find in the water which, at the time of the floods, overflowed the region of the salt-water lake lying eastward from Calcutta, a form of bacteria which at the first glance had a certain likeness to the cholera-bacilli; but, by accurate examination, they appeared somewhat larger and thicker, and their cultivation did not liquefy the gelatine."

Last summer, I had the opportunity of investigating the dejecta of two cases of severe cholera nostras, one of them fatal. In both were comma-shaped bacilli, closely resembling, in microscopic appearance, Koch's cholera-bacilli; and, in the second case, there were also large numbers of spirilla as well. As I was very anxious at that time to get cultivations of the cholera-bacillus, and as it was also possible that

these cases might be the first in an epidemic of Asiatic cholera, I spared no pains in my attempt to cultivate these organisms; but, although I made a large number of cultivations, I was quite unable to cultivate them, the same material and the same methods being employed which were afterwards perfectly successful in the cases of true Asiatic cholera. There can, therefore, be no question but that these organisms belonged to a separate species or variety, whatever the proper term may be. In

	Cholera.	Finkler.	Flügge.
Microscopic appearance.	Distinctly comma-shaped, except the small young forms. Spirilla not unfrequent, consisting of 8 to 30 turns.	Larger than cholera, not so markedly curved; spirilla rarer than in cholera, and not so long (3 to 6 turns).	Smaller than cholera; spirilla more frequent than in cholera, but curves not so marked, more angular, and number of turns not so great, nor are the turns so wide apart.
Glass-plate cultivation in nutrient jelly.	Small irregular colonies, not round, granular; liquefies gelatine comparatively slowly; may ultimately in a few days cover area of 1 millimètre.	Regular colonies; flat contour; liquefies gelatine very rapidly, so that in 30 hours it may occupy area of 1 centimètre.	Very dark, round colonies, contour regular, surface granular; liquefies more rapidly than cholera, but not so rapidly as Finkler. The early stage is not unlike a late stage of the cholera-bacillus.
Test-tube cultivations.	Air-bubble appearance liquefies in funnel-shape; may reach edge at surface in about a week; does not liquefy rapidly at lower part of track; fluid clears; highly refracting granules on side of funnel; rose-coloured deposit.	Liquefies very rapidly; in 24 hours may reach side of tube, and also extend down to bottom of the needle-track. Fluid diffused, muddy.	Early stage of this not unlike late stage of cholera, fluid somewhat more turbid, and lower part of track sooner liquid.
Potatoes.	Does not grow under 32° C. At body-temperature, forms dark brown layer.	Grows rapidly at 18° C., forming a thick greyish-yellow layer.	Does not grow on potatoes at all.

a diseased bee, I also found comma-shaped bacilli; but, unfortunately, I did not subject them to a thorough investigation, so that I have really nothing more definite to mention than the microscopic observation, which, of course, tells us nothing with reference to the question at issue.

In saliva, more especially, however, in the accumulations on the teeth, comma-shaped bacilli are frequently present—sometimes, indeed, in considerable numbers; and as here, if anywhere, the cholera-bacilli ought to be found, if they be only accidentally present in cases of Asiatic cholera, a great deal of attention has been paid to these organisms by various observers. Dr. Koch himself, in his earliest reports, states that he has paid particular attention to this matter, and that he has entirely failed to find the cholera-bacilli in the saliva, or in the accumulations on the teeth. I have, during the course of the past winter, tested my own saliva, and that of other persons, on a number of occasions, for cholera-bacilli, but have entirely failed to find any. The experiments were performed in the same way as in the case of the cholera-evacuations, by glass-plate cultivations in the nutrient jelly; and I have done, on an average, one set of experiments (from six to ten plates) every week since the beginning of December. Not only have I

failed to obtain cholera-bacilli, but I have entirely failed to grow the comma-shaped bacilli seen in the saliva. In several cases, there were numerous comma-shaped bacilli present in the material tested ; but I, nevertheless, failed to obtain cultivations of them. As regards the material employed for cultivation, I have in most instances used the same nutrient jelly as was being at the same time used successfully for the cultivation of the cholera-bacillus. This material was as nearly neutral as possible, though generally, no doubt, it was faintly alkaline ; in some cases, however, it was faintly acid ; and in several instances it was not neutralised at all, and was therefore strongly acid. Learning lately that it was supposed that the salivary comma-shaped bacilli could be cultivated in absolutely neutral jelly containing five per cent. gelatine, I have prepared some material absolutely neutralised by the use of calcium-carbonate, as suggested to me by Professor Warden ; but I have entirely failed to obtain cultivations of the comma-shaped bacilli, although considerable numbers were seen on microscopic examination. From my own experiments, therefore, I have no doubt that the comma-shaped bacilli seen in the saliva are not the same organisms as the cholera-bacilli described by Koch. The same failure to cultivate the comma-shaped bacilli of the saliva has attended attempts made by a large number of observers ; indeed, with the single exception of Dr. Klein, no one has succeeded in growing them. I shall refer to Dr. Klein's experiments in the appendix ; but I may here say that, in my opinion, they cannot, so long as they stand unconfirmed, be regarded as free from error. Professor Miller, a dental surgeon in Berlin, has made cultivations from a very large number of patients, and has entirely failed to obtain the cholera-bacillus. Indeed, in only one case, from a carious tooth, did he succeed in obtaining a cultivation of comma-shaped bacilli which liquefied gelatine ; but, on further investigation, it turns out that this organism is very closely allied to, and in all probability identical with, Finkler's bacillus. It seems to me that we have now sufficient evidence to enable us to say definitely that the cholera-bacilli are not present in saliva ; more than sufficient evidence, if we bear in mind the fact that they are always present in Asiatic cholera ; and that, therefore, if they come from the saliva, and are not peculiar to cholera, they ought to be always, or at least very frequently, found in it.

I have also, in several instances, tested the evacuations from diarrhœa by cultivation for the cholera-bacillus, with entirely negative results. I have referred before to the case of diarrhœa in Paris, in which I also obtained a negative result. Then I have tested putrefying and other liquids containing various kinds of bacteria, without obtaining any cholera-bacilli. And, further, I may bring forward my previous experience with these methods of research, extending now over several years, in which I have investigated water, air, soil, in fact all sorts of materials, in the first instance for practice, and later, with the object of making myself acquainted with the various forms of bacteria, or for other purposes; the result of this experience being that I never met with these organisms till I investigated cases of Asiatic cholera.

The answer which I then give to the second point is, that I have never met with the cholera-bacillus, except in Asiatic cholera, and that the other comma-shaped bacilli as yet described, differ markedly from it in many essential points. This statement is founded on a thorough examination of a large quantity of material.

I now pass on to the third point ; namely, the result of injection of these organisms, or their products, into animals. Numerous attempts have at various times been made to infect the lower animals with cholera-dejecta, but the results have been quite indefinite ; in fact, the natural conclusion from them is that the lower animals are not susceptible to the virus of cholera. Cholera-dejecta have been in-

jected into the stomach, small intestines, and veins of various animals without producing any effect. Mice seem to have been the only animals in which anything like positive results were obtained; but it is apparently now very doubtful whether they were really affected with the virus of cholera, or only with some form of septicæmia. Seeing then that the virus of cholera, supposing it to exist in the dejecta, is without effect on the lower animals when mixed with the dejecta, it could not be expected that if it were isolated from the other materials it would be more potent. Hence a failure to produce disease with any given bacterium which, for other reasons, might be regarded as causally connected with the disease, does not in any way prove that the said bacterium is not the virus sought for. As regards Koch's cholera-bacillus, the earlier results of injection into the small intestine, or into the veins, or of feeding the animals with pure cultivations, were also entirely negative. Thus the question remained *in statu quo*, and all that could be said was, that this failure did not demonstrate that these bacilli were not the cause of cholera, but, if anything, rather strengthened the contrary opinion, as showing that in this particular the relation of this organism to the lower animals was the same as that of the virus of cholera. The matter has not, however, been allowed to remain in this condition; numerous efforts have been made to overcome, in some way or other, the difficulty of infecting animals with cholera, and to some extent these efforts have been successful.

During an investigation on cholera, Nicati and Rietsch, being struck by the fact that the biliary secretion seemed to be diminished or arrested during an attack of cholera, thought that this might have something to do with the rapid growth of the cholera-bacilli in the intestine. They therefore tied the bile-duct in dogs, and then injected pure cultivations of cholera-bacilli into the intestine. They report that their experiments were successful in several instances, the animals suffering from diarrhœa, and dying in two or three days. The intestine after death was found to be reddened, and to contain fluid watery dejecta, resembling the rice-water material in man. They were also able to produce similar effects in guinea-pigs. Shortly afterwards Dr. Koch reported that he had been able, in some instances, to obtain like results in guinea-pigs. Apparently, however, the results were by no means constant.

When I returned from Paris, I wrote to Dr. Koch to inquire what his method of operating was, and he kindly gave me full details. The essential point was to inject the cultivation into the duodenum between the pyloric end of the stomach and the entrance of the bile-duct, the whole operation being done with thorough antiseptic precautions These precautions consist in shaving the skin of the abdomen, washing it thoroughly with watery solution of corrosive sublimate (1 in 1000), and with carbolic acid; the hands, sponges, knives, and other instruments are also thoroughly disinfected, and the wound is dressed with an antiseptic dressing. I have always, after stitching up the wound closely with silver wire and catgut, applied powdered salicylic acid in large quantity, so as to form an antiseptic crust.

The operation is by no means a difficult one, and guinea-pigs—which are the only animals I have used for these experiments—take chloroform very well. A small incision, about half an inch to the right of the middle line, extending from the lower border of the ribs downwards, exposes the lower edge of the liver and the stomach close to the pyloric end. By pulling gently on the stomach, the pyloric end and the first part of the duodenum come into view. The syringe employed was that introduced by Dr. Koch for experiments with bacteria, remarkable chiefly for the fact that the metal portions screw on to the glass, and that the washers and a portion of the piston are renewed every time the syringe is used. The syringe is

always sterilised by keeping it in an iron box at 300° Fahr. for three hours after the fresh washers and piston have been applied. In injecting into the intestine, the needle is pushed through the walls as obliquely as possible, so as to prevent regurgitation into the peritoneal cavity. As a rule, cultivations in nutrient jelly were employed. A little distilled water was boiled, and allowed to cool under protection from dust. A small quantity of the growth was then removed from the tube by means of the sterilised platinum wire, and diffused in the water. I shall now give a list of the experiments I have done.

No.	Quantity.	Age of Cultivation.	Result.
1	18 minims	7 days	Found dead next morning.
2	1 minim	13 days	No effect.
3	3 minims	Mixt. of 7 and 19 days	,, ,,
4	9 minims	8 days	Died in 4 days. This animal died on a Sunday and was not seen till the following morning, having lain in the cage with the other guinea-pigs all night. When examined, it was found that the other guinea-pigs had eaten all the abdominal organs of this animal, so that there was nothing left to investigate. The other guinea-pigs remained well.
5	,, ,,	3 days	No effect.
6	,, ,,	,, ,,	,, ,,
7	,, ,,	,, ,,	,, ,,
8	14 minims	,, ,,	,, ,,
9	18 minims	5 days	Died in 2 days. This animal had a little diarrhœa, and apparently some difficulty in breathing, and spasmodic movements before death. The intestine was found to be reddened, especially around Peyer's patches, and contained a large quantity of watery fluid. This fluid, tested by cultivation from the duodenum, the ileum, and the large intestine, contained practically a pure cultivation of cholera-bacilli. Blood dark and fluid; no bacteria in blood or internal organs.
10	,, ,,	,, ,,	No effect.
11	,, ,,	,, ,,	Died in six days. In this case, again, I had a misfortune; for, being called away just as I had sat down to make cultivations from the intestinal contents, I found, on my return, that my cat had eaten everything except the head. The cat remained quite well.
12	,, ,,	,, ,,	No effect.
13	,, ,,	8 days	Died in two days. Precisely the same conditions as in No 9. In one Peyer's patch there was considerable hæmorrhage.
14	,, ,,	,, ,,	No effect.
15	½ minim	13 days	,, ,,
16	18 minims	5 days	,, ,,
17	,, ,,	,, ,,	No effect. In this case, a large part of the fluid was distinctly seen to pass into the stomach through the pylorus; and this was the case, I think, in several of the other experiments.

The result of these experiments is not at all conclusive; nevertheless, in two instances, Nos. 9 and 13, a definite effect was produced. In case No. 1, the animal probably died of shock; it was the first time I had performed the operation, and I did not do it very well. As to cases 4 and 11 I can say nothing, as I could not investigate them, but I think, from the symptoms, that No. 4 was killed by the cholera-bacilli. Nos. 9 and 13 were, as I have said, quite definite. In both these animals there was diarrhœa, though this symptom was not very marked; but the appearance of the intestines after death was very like that seen in Asiatic cholera. It was evident that these animals had been killed by the cholera-bacilli; and the result is, in so far, important and interesting, as showing that when the cholera-bacilli

do produce an effect, their chief action is in the intestines, and they do not penetrate into the blood, resembling, in this respect, the probable action of the virus of cholera.

As regards these cases, I am perfectly satisfied that the animals did not die of any form of septicæmia, and I made a particularly thorough investigation of this point in the case of No. 13, as I then knew that Dr. Klein was attributing Dr. Koch's successes to septicæmia.

As I have said, in neither case were any organisms found in the blood or internal organs, whether by microscopic examination after staining, or by cultivation. In both cases I inoculated other animals with the blood from the heart, without producing any effect. In none of the animals was there any trace of peritonitis. Why these two animals alone were affected, I cannot say. I have injected some of the guinea-pigs after they had fed, and others after they had been deprived of food for some hours. I have used cultivations of different ages, and cultivations in different materials, without any difference in effect being produced.

The only thing in which the two successful cases differed from most of the others, was that the bacilli were growing in jelly containing three per cent. of peptone, while, in most of the other cases, one per cent. of peptone was employed; but a subsequent attempt with jelly containing three per cent. of peptone, and also with jelly containing four per cent. of peptone, gave negative results. Hence the effects did not depend on the amount of peptone in the cultivating medium, and Dr. Koch, whom I consulted on the matter, agreed with me on this point.

I have not continued these experiments of late, partly because I expect that Dr. Koch will shortly throw much new light on this part of the subject, and partly on account of the limited number of animals on which one is allowed to experiment.

I have also injected a syringeful of a pure cultivation of these organisms in nutrient jelly into each of four mice. On one occasion, for two of the mice, a cultivation nine days old was employed, and both mice died in about six hours. In about an hour, they seemed sleepy and less lively than usual, sitting crouched up in a corner, and breathing rapidly. For at least an hour before death, however, they lay on their sides breathing very slowly, and apparently with great difficulty; in fact, rather gasping than breathing.

On another occasion, the other two mice were injected with a cultivation three weeks old. One of these remained unaffected, the other died in thirty-six hours. On *post mortem* examination, the blood was dark and fluid, and the intestines contained very liquid fæces. Cultivations were made from the blood of the heart, and from the contents of the small intestine. In both instances, large numbers of cholera-bacilli developed. The result in the first two cases was, I think, clearly due to a chemical poison; that in the last, to growth of the organisms in the blood. It is interesting, with regard to the last case, that the organisms had found their way into the intestine, and grown there in large numbers. I have not yet examined the wall of the intestine microscopically.

It is held by many that the virus of cholera produces a chemical poison which is absorbed from the intestine into the blood, and gives rise to the symptoms of the disease. This is a very probable view. Dr. Vincent Richards (*Indian Medical Gazette*, April 1884) found that the administration of large quantities of the contents of the intestines of cholera-cases to pigs was, in some instances, followed by rapid death of the animal, apparently from a chemical poison. He mentions five experiments, in three of which the result was negative (in only two, however, was choleraic material from man employed); in the other two, the result was positive, one animal dying in 2 hours, 50 minutes, and the other in 1 hour, 38 minutes. From these experiments, he

draws various sweeping conclusions, but there is a complete absence of control-experiments, and, as I have just mentioned, the result in three instances was quite negative. Hence no conclusion can be come to as to whether or not it was the cholera-virus which produced the poison that killed the two animals. Before I knew that the experiments were so incomplete, having only seen a notice of them in one of the English medical papers, I performed the following two experiments. My idea was that, if the poison in Dr. Richards' experiments was produced by the virus of cholera, and if the cholera-bacilli were that virus, the same results ought to be obtained by the administration of a fluid in which cholera-bacilli had grown, as by the administration of cholera-stools. Therefore, having, through the kindness of Mr. Horsley, obtained a young pig, I on two occasions made it swallow a large quantity of meat-infusion, containing peptone, in which cholera-bacilli were actively growing. On the first occasion, I used three and a half ounces of a cultivation five days old, in meat-infusion containing 1 per cent. of peptone; a week later, I used about twelve ounces of a cultivation three days old, in meat-infusion containing 4 per cent. of peptone. In neither case were any symptoms produced. Having by that time made myself acquainted with Dr. Richards' paper, I saw how incomplete his experiments were, and I did not, therefore, continue the investigation; for it was quite clear that, by working with these cultivations in the first instance, I was beginning at the wrong end, as the investigations must first be thoroughly carried out with cholera-evacuations and other materials for control, before one had any basis to go upon; and this I could not do. Nevertheless, I have thought it right to mention the facts, though I do not think that any conclusions can be drawn from them.

Nicati and Rietsch (*Comptes Rendus*, vol. xcix, pp. 928-9) found that, by filtering fluids in which cholera-bacilli had been growing, and then injecting the filtered liquid into the veins of animals, serious symptoms were produced. In a first series of experiments, they found that the results were diarrhœa, vomiting, general depression, and recovery in an hour. In a second series, there were respiratory trouble, vomiting, paralysis of legs, and death. In a dog, there was found, after death, extensive ecchymosis in the duodenum; the bladder was empty; the cortical part of the kidney was much injected; the blood was dark, no clots, etc. They state that this result will not be obtained unless the cultivations be at least eight days old; recent cultivations are quite inactive; subcutaneous injection of the material produced no result. The age of my cultivations may be a possible source of fallacy in the experiments on the pig mentioned above; for, in the case of the two mice previously referred to, in which the cultivations were nine days old, death obviously resulted from a chemical poison.

The result of my experiments on animals is, then, the following. In two instances, guinea-pigs died undoubtedly as the result of the growth of the cholera-bacilli in their intestines; they certainly did not die of septicæmia. I have, in Figure 5, given a drawing of bodies which were found in the large intestines of these animals, along with other bacteria, straight and curved. The woodcut shows them thicker than natural. I think it most probable that these bodies are the cholera-bacilli, which were found, on cultivation, to be present in enormous numbers, because there were no other markedly curved organisms present, and because they seemed to show all gradations between small slightly curved rods, and the large coiled bodies shown in the drawing. It is, however, only right to say that the gentleman who made the drawings for me thought that these bodies were minute entozoa, but this view did not seem to me probable. The microscopic appearance is, however, of no consequence; the essential fact being that, on cultivation, I found that almost all the colonies which developed on the plates, were colonies of cholera-

bacilli, showing that these were present in the intestine in enormous numbers. I should also state that I have made cultivations from the intestinal contents, and from the dejecta, of healthy guinea-pigs, and have never found cholera-bacilli. Why the other animals escaped after the injection, I cannot explain, but somewhat similar facts have been obtained by other observers. As remarked in regard to case No. 17, the fluid was seen to pass upwards into the stomach, as well as downwards into the duodenum; and it is quite possible that this acid material, passing immediately afterwards from the stomach into the duodenum, killed the bacilli which had not entered the stomach. As regards mice, in two instances death occurred in a few hours after subcutaneous injection of a cultivation, as the result, apparently, of absorption of a chemical poison from the material injected; and, in one instance, the cholera-bacilli grew in the blood of the animal, passed into the intestine, and killed the animal.

Conclusions: Diagnostic Value.—If, now, we consider carefully the meaning of the foregoing facts, it will be evident that the discovery of the cholera-bacillus is a most valuable addition to our knowledge, quite apart from the conclusions which may be come to as regards its causal connection with Asiatic cholera. For, in the first place, it seems to be constantly present in Asiatic cholera. I do not mean to assert that the cases I have examined are sufficient to prove this, but they are, nevertheless, very striking, for I had not selected the cases in any way. I did not say, "This seems a typical case of the disease, I will examine it," or "This does not seem a good case, I will not have material from it;" I was glad to take all the material offered me, without reference to whether it came from a typical case or not, the only stipulation being that it should come from a recent case. Therefore the foregoing cases, though few in number, afford very strong confirmatory evidence of the statement that these organisms are always present in Asiatic cholera. This is a fact which is, I think, hardly disputed. In the second place, this organism has never yet been found anywhere else than in Asiatic cholera. I have mentioned a large amount of evidence in support of this statement, and, for my own part, I am now thoroughly convinced that it is correct. As I have pointed out, various organisms with somewhat similar morphological characters have lately been described, but accurate examination has shown that these are different from the cholera-bacillus, and can be readily distinguished from it. Hence the converse of these propositions necessarily follows, namely, that, if the cholera-bacillus be found in dejecta, these dejecta must have come from a patient suffering from Asiatic cholera; in other words, the presence of this bacillus may be used as a means of diagnosing Asiatic cholera. The only fact[1] which could be brought forward against this view is that Dr. Klein states that, by acclimatisation, he is able after a time to cultivate the comma-shaped bacillus found in saliva in the same material as is employed for the cultivation of the cholera-bacillus, and that these cultivations are identical with those of the cholera-bacillus in many respects. I shall refer in detail in the appendix to Dr. Klein's statements with regard to the salivary bacillus. Let us, however, suppose for a moment that they are correct, and let us further suppose that the cultivations obtained by acclimatisation are identical with those of the cholera-bacillus, not merely in many respects, but in all respects: how would that affect the diagnostic value of the cholera-bacillus? It would not interfere with its value as a diagnostic sign at all; for Dr. Klein states that, *in the first instance*, the salivary comma-bacillus will *not* grow in the alkaline nutrient jelly used for

[1] I do not here take into account the statements made by Dr. Klein in last week's JOURNAL, for these require confirmation before they can be accepted. The experiments are unfinished and uncontrolled, and have, therefore, been too hastily published.

the cultivation of the cholera-bacillus; it must be acclimatised. If, therefore, the comma-shaped bacillus constantly present in Asiatic cholera be the same organism as the salivary bacillus, it must have been "acclimatised," as the result of the choleraic process, for it will grow in the first instance in the alkaline nutrient jelly. But the absence of the cholera-bacillus in diarrhœa from other causes, and under other circumstances, proves that this "acclimatisation" does not occur in other diseases than in Asiatic cholera. Hence, if, *in the first instance*, cultivations of cholera-bacilli be obtained in the alkaline nutrient jelly from dejecta, these dejecta must have come from a case of Asiatic cholera; this conclusion being necessary, as I have just pointed out, whether one holds that the cholera-bacillus is causally related to cholera, or that it is merely the salivary comma-bacillus "acclimatised" by the choleraic process. I feel certain, therefore, that I am not in any way misleading the members of the Association when I state that the presence of the cholera-bacillus in dejecta may be held to be a diagnostic sign of Asiatic cholera.

The importance of this discovery cannot, I think, be overrated; for it is probably only at the commencement of an epidemic that much can be done to arrest the spread of the disease; and if we can with certainty diagnose the first case as true Asiatic cholera, an immense point is gained. In any suspicious case, the patient can be isolated, his dejecta thoroughly disinfected, and all the necessary precautionary measures adopted; while, in the meantime, it is being ascertained whether or not it is a case of true Asiatic cholera. Thirty-six to forty-eight hours would suffice for this purpose; because, in thirty-six hours, the colonies on the glass plates are visible under a low power of the microscope, and their characteristics can be studied. I venture to think that, if Koch's work on cholera lead to nothing more than this, it is an achievement for which he deserves the very highest praise. The great importance of this matter has been insisted on by Dr. Koch, and has been realised by the German Government. During the past few months, over 100 medical men from various parts of Germany, and from other countries, have received instruction from Dr. Koch in the methods of cultivating and distinguishing the cholera-bacillus, and there are now in almost every town in Germany men able at once to ascertain, with regard to any suspicious case, whether or not it is a case of Asiatic cholera. Surely some steps ought to be taken in this country to enable our medical officers of health to acquire like information.

Causal Connection.—But if we look more closely at the facts, it will be evident that there is fair ground for thinking that the cholera-bacillus is, in some way or other, causally connected with the disease. Let us take the two main points which have been discussed in detail in the foregoing paper. In the first place, we have seen that the cholera-bacilli are always present in Asiatic cholera in the early period of the disease. Wherever cases of Asiatic cholera have been thoroughly examined, whether in India, in Egypt, in France, in Italy, or in Spain, these bacilli have been found. Wherever the virus of this disease goes, the bacillus goes; when the virus disappears, the bacillus disappears. The virus has never been found to produce Asiatic cholera without the cholera-bacillus appearing at the same time; the two evidently go hand in hand. In the second place, this bacillus has never yet been found in other diseases, or in places where any connection with Asiatic cholera is out of the question. And yet, if it be only accidentally present in cholera, it follows, from the fact that it is constantly present in that disease, that it ought to be very widely distributed throughout the world; and ought, therefore, to be readily found quite apart from Asiatic cholera. This, however, is not the case, as I have shown at length in this report. And it must be remembered that it is not one investigator alone who has failed to find these organisms

apart from Asiatic cholera ; but it is, I might almost say hundreds, at any rate not much under two hundred, and probably more, who have been searching for it diligently for months without success. When one considers the immense amount and variety of material which must, therefore, have been carefully examined with negative results, the statement, that this bacillus is limited to Asiatic cholera, must, I think, be taken as sufficiently proved. The only investigator of note whose results seem, at first sight, to lead to an opposite conclusion is Dr. Klein ; but, in the appendix, I shall point out facts in relation to his research which diminish very materially the importance to be attached to it.

Now, let us ask what can be the meaning of these two facts ; and, in doing so, we may leave out of consideration for the present the minor points, such as the distribution of the bacilli in the intestinal canal, their relation to the wall of the intestine, etc. Though these may act as additional arguments for or against the view of the causal connection of these bacilli with Asiatic cholera, yet, in the main, they have reference rather to the mode of action of the organism, supposing it to be the cause of the disease. Dr. Koch has given in his report the only three possible hypotheses which can be formed from these facts.

In the first place, one might say that the choleraic process favours the growth of these bacilli, by providing a medium in which they can grow more rapidly than other forms of bacteria. But this hypothesis would imply that these organisms are normally very widely distributed throughout the world, which, as we have seen, is not the case. It is absurd, and, indeed, it is against the facts, to suppose that cholera-bacilli are constantly present in every individual, and yet are in such small numbers that they cannot be detected, for we know that cholera-dejecta are not the only soil on which these organisms can grow luxuriantly ; they grow readily in all sorts of putrescible materials, and if they are constantly present in every individual, they must of necessity often meet with soil, either inside or outside the body, in which they can grow well. It is difficult, also, to see how they can prolong their existence if they can only grow with difficulty, and in small numbers, except when the patient is suffering from Asiatic cholera. Nor is it possible to imagine what their function in the economy of Nature could be under these circumstances, for every one of these bacteria has its proper function. Besides, considering the enormous amount of material that has now been examined for them, they would certainly have been found by this time. This hypothesis may, therefore, be dismissed, and the other two considered.

In the first place, it might be said that, as the result of the choleraic process, some common and well known form of organism changes its characters, and becomes converted into the cholera-bacillus ; or, in the second place, it must be concluded that the cholera-bacillus is, in some way or other, causally connected with the disease.

The first conception is quite inadmissible in the present state of our knowledge. We are here brought face to face with the question of the conversion of one form of micro-organism into another. This, though possible on the evolution-theory, is contrary to all carefully observed facts with regard to bacteria, and, for this reason, it must be proved beyond a doubt before it can be accepted in any case. The evolution-theory is all very well, but it is still only a theory, however probable, and it must not be put in opposition to facts. But, even on the evolution-theory, it is hardly possible to admit this view ; for change on the evolution-theory—such change as involves complete loss of original characters and acquisition of new ones—requires a long time, a great number of generations.

Now what is a generation ? In the case of the higher plants, it would

be reckoned from seed to seed. The formation of each new cell does not imply a new generation. And so, in the case of the spore-bearing bacteria, I should be inclined to reckon the generation from spore to spore, and not the mere division of the rods, which seems to me more comparable to the formation of new cells in the same generation. But if one reckons the whole cycle of changes from the sprouting of one spore to the formation of new spores as a generation, then the idea that one spore-bearing bacterium can be converted into another in a few hours or days, is quite untenable.

In the case of the cholera-bacillus, I doubt whether the complete cycle is yet known ; but, so far as our information goes, it probably extends from the early stage, when the bacillus is almost straight, to the curved stage, and thence to the spirillar stage. But this cycle takes a considerable time ; and the idea that a sufficient number of these cycles occur in a single case of cholera to convert one organism into another is quite out of the question. But, even granting that the formation of each new cell is a generation, there is still not time for conversion of one organism into another. Take case No. 2 as an example. Here, in twenty-four hours from the first symptoms of the disease, there were already innumerable myriads of cholera-bacilli in the dejecta ; hence, on the view we are considering, this change must have occurred a considerable time previously ; in fact, within a very few hours of the commencement of the choleraic process, in a period of time too short, so far as we can judge, for the occurrence of change. And then, again, the cholera-bacillus can be grown through a large number of generations extending over months, and it does not show the slightest evidence of change. My own cultivations retain precisely the same characters as when they were first obtained five months ago. And yet, if this organism be so very unstable, that a few hours in a cholera-patient suffice to change its characters, one would expect that it would again revert to its original characters, more especially as it has been grown under a great variety of different conditions. The attenuation of anthrax-bacilli is not a case in point, as there there is no alteration in form and other characters, but only loss of pathogenic properties. The only instance which could be adduced is Buchner's experiments, in which he thought that he had converted the innocent hay-bacillus into the virulent bacillus-anthracis, and *vice versâ ;* but these experiments have been repeated by various observers, amongst others by Dr. Klein, and the conclusion come to was that Buchner's results were due to accidental contamination of his cultivations, and not to conversion of one organism into another ; and Dr. Klein has published a number of observations on other bacteria to show that a change of this kind does not occur. But I need not refer further to this view, against which all experimental evidence is unanimous, and in support of which no reliable facts are known.

We are then left with the other alternative, namely, that, so far as our present knowledge goes, there is no other probable explanation of these two leading facts than that the cholera-bacillus is, in some way or other, causally connected with Asiatic cholera. There are, moreover, various other facts which favour this view, in addition to the difficulty of finding any other probable explanation of the exclusive association of this organism with Asiatic cholera.

According to Dr. Koch, these organisms are present in greatest numbers in the most acute cases of the disease, and at an early stage. In the cases previously mentioned, it will be seen that they were most numerous in the dejecta from a case only ill twenty-four hours, and least numerous where the patient had been ill for four days and was recovering. Again, the experiments on animals show that, when this organism can grow in the intestine, it sets up a morbid process similar to cholera. In my own experiments, two definite cases of this kind occurred, and I have previously hinted that we will shortly have very

striking evidence from Dr. Koch on this point. In fact, it seems as if he had found out why it is that the injections performed in the way I have previously described are so uncertain; and apparently, as I understand, he can now infect the animals with certainty with pure cultivations of the cholera-bacillus. I need not, therefore, enter into this matter of experiments on animals, for Dr. Koch's further report will be published very soon. Again, this organism is remarkable for the rapidity with which it is killed by drying; and Mr. Macnamara mentions, in his work on *Asiatic Cholera*, that, during the epidemic spread of the disease, districts suffering from drought were entirely passed over, although small-pox and other epidemics were raging freely in them. Again, Mr. Macnamara states, as the result of his very precise observations, that dilute acids—the gastric juice, for example—kills the virus of cholera; gastric juice, as we have seen, kills the cholera-bacillus very rapidly. He also came to the conclusion that decomposition destroys the virus of cholera, and Dr. Koch came to the same conclusion with regard to the cholera-bacillus. Then the way in which epidemics disappear from Europe would imply that the virus cannot be very tenacious of vitality; the cholera-bacillus has no spore or resting-stage, and soon dies when it has insufficient nutriment.

But, while there appears to be every reason for believing that without this bacillus Asiatic cholera could not occur, it is evident that this disease is one in which other factors play a most important part. It remains to be ascertained whether this bacillus stands on the same footing as other pathogenic bacteria—say, the bacillus anthracis. Inject anthrax-bacilli into an animal, and they will practically, with certainty, cause anthrax. Is the cholera-bacillus equally potent? May there not be some truth in Pettenkofer's views, and may not this bacillus only acquire its virulence in suitable soil outside the body? What is the meaning of the epidemic outbreaks of this disease, and why is it at times endemic without being epidemic? In fact, what other conditions come into play, and what is the importance of the share they take in the causation of the disease? That other conditions do play a very important part, is evident from the whole history of cholera, and I can only explain the great, and apparently hopeless, diversity of opinion among Indian observers, as to the contagionsness of the disease, by supposing that one observer has paid special attention to one set of conditions, and that another must have been specially struck by other conditions. These are the points which, it seems to me, now urgently require investigation; for, till some conclusion is come to as to all the necessary conditions concerned in the process, we can hardly expect to be able to decide on the best and most practicable means of prevention and cure, although, no doubt, the knowledge gained by Dr. Koch's work must greatly advance matters.

Appendix.

It is not necessary for me to enter at length into Dr. Koch's paper, as his complete report was published in this Journal last year; but I may sum up his statements as follows.

1. The cholera-bacillus is a distinct species of micro-organism, having marked characteristics which distinguish it from all other known organisms. In this connection, I may mention that not only does Dr. Koch lay particular stress on the description of the cultivation-characters of these organisms, but he states distinctly, in more than one place, that the microscope alone will not suffice for their detection in most cases, but that the culture-test must be employed as well.

2. This bacillus is always present in Asiatic cholera.

3. It is the only form which is constantly present there and nowhere else.

4. It is present in greatest numbers in acute and uncomplicated cases.

5. It is present in the parts most affected.

6. It is never present in other diseases, or in healthy persons; nor has it been found outside the body when there was no cholera in the neighbourhood.

7. No other conclusion can be arrived at than that these bacilli are the cause of cholera. (The other hypotheses have been already discussed.)

8. Although, by experiments on animals, direct evidence that the cholera-bacillus is the cause of cholera has not yet been obtained, there are various observations which are almost as good as experiments on man, and which go to support the idea of their causal connection.

9. The natural history of the disease corresponds with the various characteristics of this organism.

In a later paper, Koch states that he has again taken up the experiments on animals, and that he has succeeded in causing the death of the animals, with *post mortem* appearances like those of cholera, by injection of pure cultivations of the cholera-bacilli. His forthcoming report will be awaited with much interest.

Van Ermengem was able to confirm Dr. Koch's statements in regard to the constant presence of these organisms in Asiatic cholera. He found them in thirty-four specimens of dejecta from cholera-patients, and in eight *post mortem* cases. He found that the number present corresponded with the stage of the disease. In two extremely acute cases, they were present almost in pure cultivations. In one case in the algide stage (cholera algida), only very few were seen on microscopical examination; but, on cultivation, enormous numbers were found. He was also able to infect animals with the cholera-bacilli.[1]

Dr. Victor Babes (Virchow's *Archiv*, vol. xcix, 1885) investigated ten recent and five older cases in Paris. He found the same striking difference between the results of the microscopical examination and the results of cultivation to which I have previously alluded; and, as his experience very strikingly illustrates the untrustworthiness of microscopic examination alone, I shall quote his words. He says: "In all of these cases, I examined the intestinal contents, in the first instance, with the microscope. Only once did I find a completely characteristic appearance; in this instance, there were in every

[1] Since the above was written, I have received Dr. Van Ermengem's work, entitled *Recherches sur le Microbe du Choléra Asiatique*, a work which deserves careful study by anyone interested in this subject. He undertook the investigation on behalf of the Belgian Government, without being prejudiced in any way, and the result of his work is complete confirmation of Dr. Koch's facts and views.

field of the microscope countless comma-bacilli, here and there united so as to form wavy lines. In five cases, there were almost no other forms of bacteria, except comma-bacilli in considerable numbers, in the flakes in the intestinal contents. In the other cases, on the contrary, the comma-bacilli were mixed with the ordinary intestinal bacilli, or with a peculiar bacterium composed of two almost lancet-shaped members. The comma-bacilli were often in such small numbers, and their form so little characteristic, that I should not like to conclude from such an appearance that I had to do with cholera. In two of the older cases, I found absolutely nothing which I could recognise as comma-bacilli. The state of matters is, however, quite different when one employs portions of the dejecta, or the whitish masses lying close to the mucous membrane, especially over Peyer's patches, for the preparation of cultivations. By this procedure, I obtained, in nine of the ten recent cases, the characteristic cultivations of the comma-bacillus; and the possibility is not excluded that the negative result in one case was due to an error in the experiment. In two of the five older cases (six to ten days old), I was, however, unable to discover any colonies of comma-bacilli, even although in one case I prepared a number of plates."

He agrees with Koch and Ermengem in regarding Finkler's comma-bacillus and the comma-bacillus of the saliva as quite different organisms from the cholera-bacillus. He finds that the cholera-bacilli are difficult to stain in sections.

Nicati and Rietsch also found the cholera-bacilli in cases of cholera, and succeeded in infecting animals with them. In the case of dogs, they first ligatured the bile-duct, and then injected pure cultivations of these bacilli into the intestine. In the case of guinea-pigs, they also succeeded, even without ligaturing the duct. I have already referred to their experiments, and, therefore, I need not mention them further.

Schottelius (*Deutsche Medicinische Wochenschrift*, April 2nd, 1885) states, as his opinion, that, for the exact demonstration of cholera-bacilli, the culture-test cannot be dispensed with. He says: "This method, according to my experience, gives positive results, without exception, even in cases in which no comma-bacilli could with certainty be detected by the microscope in dejecta, of which numerous specimens had been microscopically examined." He proposes, however, a more rapid way of arriving at a diagnosis than by the plate-cultivation test, which requires about thirty-six hours. He mixes a considerable quantity—100 to 200 cubic centimètres—of the suspected dejecta with 250 to 500 cubic centimètres of faintly alkaline meat-infusion. This mixture is well shaken, and placed in a tall vessel, which is put in a warm place—if possible, about the temperature of the human body, and is allowed to stand for about twelve hours. The main mass of the dejecta falls to the bottom of the glass, but the cholera-bacilli, which require oxygen, collect at the surface, and multiply with great rapidity. Microscopic specimens can then be made of the upper layer of fluid, and, if the case be one of cholera, almost a pure cultivation of cholera-bacilli will be obtained. Of course, this method alone is not sufficient, because other comma-bacilli, such as Finkler's, might be present, which might also accumulate and grow at the surface, and it would require a large amount of experience to be able to distinguish these from the cholera-organism by the microscopic appearance alone. Schottelius himself does not propose to supersede the glass-plate culture-test by the above method.

I need not mention further the confirmatory evidence in favour of Koch's statement that the cholera-bacilli are always present in cases of cholera, nor need I refer to the other evidence in favour of the second point, namely, that they are never present elsewhere; but I shall now mention shortly the chief researches which are supposed to

tell against Dr. Koch's views. It will be seen that no unanimity exists among these investigators as to the facts; they only agree on the one point, that they think Dr. Koch's views are wrong.

The French Commission were unable to discover any micro-organisms peculiar to cholera in the dejecta. They saw bodies in the blood which they thought were micro-organisms, but they were unable to cultivate them. It turns out, however, that the bodies which they saw in the blood were not micro-organisms.[2]

Finkler and Prior cultivated comma-shaped bacilli from the dejecta obtained from cases of cholera nostras at Bonn, which they thought to be the same as the cholera-bacilli described by Dr. Koch. It has now been satisfactorily demonstrated that these organisms are not the same as the cholera-bacilli, though somewhat resembling them in microscopic appearance. As I have already described the characteristics of Finkler's organism, I need not again refer to them here.

Dr. Timothy Lewis drew attention to the presence in the saliva of comma-shaped bacilli resembling the cholera-bacilli, but he did not apply the culture-test to them. It has since been shown that these organisms are not the same as Dr. Koch's cholera-bacilli.

Dr. Emmerich (see *Deutsche Medicinische Wochenschrift*, No. 50, 1884) found, by cultivation, a short thick bacterium in the blood and internal organs in cases of Asiatic cholera, which, he thinks, has a better right to be looked upon as causally connected with the disease than have Koch's cholera-bacilli. This research has been criticised at length by Professor Flügge in the *Deutsche Medicinische Wochenschrift* for January 8th, 1885; and I need only repeat one or two of the points in Professor Flügge's criticism, which seem to me to be justified. Emmerich considers that the reason why other observers have failed to find micro-organisms in the blood and tissues is, that too few cultivations have been made in each instance. If, however, only a certain proportion of the tubes show growth, it would imply, in Flügge's opinion, the presence of relatively very few organisms in the whole circulating blood, while on Emmerich's view the blood is the seat of the disease, and, therefore, ought to contain large numbers of organisms. Then Emmerich leaves out of sight the possible accidental contamination of some of these tubes during the process of inoculation, and also the possibility that the organisms may have penetrated into the blood and organs after death. Flügge further points out that Emmerich did not employ the glass plate cultivations in Naples, but merely inoculated tubes, and took them back to Munich, where the plate-cultivations were first made. This was, of course, a totally inadequate method. Dr. Emmerich apparently saw the weakness of this method, but considers the objection invalid, because it was the same organism which developed in each tube. But Professor Flügge points out that the form and mode of growth of these organisms on nutrient jelly is by no means characteristic, and that many of the commonest accidental impurities of cultivations are alike, or very similar, in their form and mode of growth. Emmerich found that, when he injected cultivations of these organisms into guinea-pigs—into their intestine, lungs, or subcutaneously—an affection of the small intestine ensued, corresponding in severity to the quantity of material injected. There was either simple catarrh, or exudation with swelling of Peyer's patches, or extensive ecchymoses and formation of ulcers, which in some cases led to perforation. In the cæcum and large intestine, there were also, at times, extensive ecchymoses. The peritoneum was injected, mesenteric glands swollen, spleen small and soft. Flügge points out, in connection with this, that these are not the *post mortem*

[2] In the *Bulletin de l'Académie de Méd. de Paris*, August, 1884, will be found a communication from M. Strauss on the cholera-bacillus. He does not express a definite opinion one way or the other, but seems rather to incline to the view that Dr. Koch has not proved his point.

appearances of Asiatic cholera in man; that Emmerich evidently only obtained it with a certain proportion of the cultivations which he brought from Naples; and that similar results follow the injection of a bacillus which has nothing to do with cholera, and which Dr. Kreibohm obtained in Flügge's laboratory from human sputum and saliva. I need not pursue the criticism of Emmerich's investigations further, more especially as most other observers who have worked out the matter are agreed that no micro-organisms are present in the blood or internal organs in Asiatic cholera; but I may end by quoting one sentence from Flügge's criticism. He says: "As a matter of fact, the state of matters is this, that Emmerich could have obtained all his results—his cultivations, the characteristics of the organisms cultivated, and the infection of animals—if he had investigated any dead body, not too long dead, which had nothing whatever to do with Asiatic cholera."

I must now pass on to Dr. Klein's statements, based on the result of the researches of the English Commission, and also of his own work since the return of the Commission. This is by far the most complete of the researches which are opposed to Dr. Koch's views, and both on account of Dr. Klein's high reputation and of the immense importance of the subject, it requires careful and thorough criticism. This research is stated by Dr. J. M. Cuningham, Sanitary Commissioner with the Government of India, to be "entirely subversive of the statements advanced by Professor Koch, as to the so-called 'comma-bacillus being the cause of cholera.'" This is a most serious conclusion, and being the view taken by a high authority in India and being, nevertheless, opposed to the weight of evidence, it is the more necessary to analyse Dr. Klein's statements very carefully, so as to be certain that there has been no possibility of error.

In the preliminary report of the English Commission, dated November 27th, 1884 (British Medical Journal, January 3rd, 1885), the first statement runs as follows. "The statement of Koch that 'comma-bacilli' are present only in the intestines of persons suffering from, or dead of cholera, is not in accordance with the facts, since 'comma-bacilli' occur also in other diseases of the intestines, for example, epidemic diarrhœa, dysentery, and in intestinal catarrh associated with phthisis." Now, if by the expression "comma-bacilli" is meant cholera-bacilli, and it ought to mean this, otherwise it is not an argument, this statement, if correct, is, as Dr. Cuningham puts it, "entirely subversive of the statements advanced by Professor Koch as to the so-called 'comma-bacillus' being the cause of cholera." And this statement is not only subversive of the view that the cholera-bacillus is the cause of cholera, but also renders it impossible to consider the presence of this bacillus as diagnostic of Asiatic cholera; hence, doubtless, the reason why no mention is made of this most important fact in the reports of the English Commission. This statement, if correct, would further imply great carelessness on Dr. Koch's part, in not having ascertained this fact during the months which he spent on his investigation, a fact apparently readily made out in a few weeks by the English Commission.

At the meeting at the Royal Society, on February 5th (British Medical Journal, February 7th, 1885) Dr. Klein repeated and extended this statement. He there said "Koch overlooked the fact that 'comma-bacilli' occur in other intestinal diseases, in the mouths of healthy persons, and, as shown recently, even in some common articles of food (by Dr. Deneke in stale cheese)." And at the meeting of the Royal Medical and Chirurgical Society, on March 24th, the same statements were repeated without any qualification whatever. When, however, we call to mind that similar materials were thoroughly examined by Dr. Koch in India, with an entirely negative result, and when the negative results obtained by other observers, as described in the preceding pages, are taken into consideration, the

question naturally arises whether this statement may not be really founded on a misapprehension of what Dr. Koch meant by cholera-bacilli. If by "comma-bacilli" Dr. Klein meant "comma-shaped bacilli," and therefore not necessarily cholera-bacilli; if, in other words, he relied on microscopic appearance alone, and not on the cultivation-characters for the determination of cholera-bacilli, the whole discrepancy is explained. It turns out now that this was really the case; for, in the BRITISH MEDICAL JOURNAL of April 4th, 1885, Dr. Klein publishes "some remarks on the present state of our knowledge of the comma-bacilli of Koch," in which he devotes a considerable amount of space to the attempt to show that the discovery of the comma-bacilli, "their description and their specific relation to cholera, were asserted by microscopic examination only," and in which he tells us that "there is nowhere (in Dr. Koch's reports) a word of a culture-test."

I was able, in a letter in the JOURNAL on April 11th, to show that, in this supposition, Dr. Klein was entirely wrong, and to this I must refer the reader. I only quote Dr. Klein's statements here, to show the view which he held as to the methods used by Dr. Koch in distinguishing the cholera-bacillus. Thinking that Koch discovered, described, and asserted the specific relation between cholera and these bacilli by the microscopic appearance alone, the English commission naturally proceeded to examine other materials by the microscope alone, and, finding comma-shaped bacilli, looked on this result as entirely subversive of Dr. Koch's statements. Had Dr. Klein not considered that the microscopic examination was sufficient, he could hardly have referred to Deneke's cheese-spirilla in the passage I have quoted from the meeting at the Royal Society, for Dr. Deneke's paper is entitled "On a New Form of Bacterium resembling the Cholera-Spirilla," and in it Deneke points out how this new bacterium may be distinguished from the cholera-bacillus. Regarding this as the only explanation of Dr. Klein's statements, I suggested it at the meeting at the Royal Medical and Chirurgical Society, and further suggested that Dr. Klein should tell us in what cases the results were obtained by microscopical examination alone, and in what cases the statement rested on culture-tests as well. The result of this was that, in the abstract of his remarks, published three days later (BRITISH MEDICAL JOURNAL, March 28th, 1885), he says "comma-bacilli of various species have been discovered in other diseases of the alimentary canal, in the fluid of the mouth of normal persons (Lewis), and in old cheese (Deneke). The comma-bacilli found by Finkler and Prior in cholera nostras, differ in mode of growth from Koch's comma-bacilli of cholera; so do those found in diarrhœa due to other causes; but those of the fluid of the mouth are identical with Koch's comma-bacilli in many respects." And, in the later paper, to which I have already referred (BRITISH MEDICAL JOURNAL, April 4th, 1885), in which Dr. Klein gives his views as to "the precise position with regard to the comma-bacilli of Koch," no mention whatever is made of the comma-bacilli found in other diseases of the intestine. In reality, the facts stated as result No. 1 of the preliminary report, instead of being "entirely subversive" of Dr. Koch's statements, do not affect the question at all, for the simple reason that the bacilli found in these cases were not cholera-bacilli.

The only matter which Dr. Klein now brings forward against the cardinal point of Koch's research (that the cholera-bacilli are found only in Asiatic cholera), is the case of the salivary bacillus.[3] This was

[3] In the BRITISH MEDICAL JOURNAL for May 16th, Dr. Klein states that, in the cæcum of normal guinea-pigs, one will find "the typical comma-bacilli of Koch." I have already investigated this matter, and I can only say that, in my experience, this is not the case. I see that here again Dr. Klein speaks of the appearances under the microscope, so that I presume he has fallen into the same error as that

not mentioned in the preliminary report at all, but was first spoken of before the Royal Society. At the meeting at the Royal Medical and Chirurgical Society, I referred to the failure, on the part of numerous observers, to cultivate the comma-shaped bacilli of the saliva, although the same material and methods were used as were being successfully employed for the cultivation of the cholera-bacilli; and I also said that Dr. Klein was the only observer who thought that he had succeeded in cultivating them. Dr. Klein then stated that the salivary comma-bacilli would not grow on the same soil as was used for the cultivation of the cholera-bacilli, on which I naturally suggested that that proved that they were not the same organisms. To this view Dr. Klein assented.

In the British Medical Journal of March 28th, 1885, Dr. Klein tells us that he obtains his cultivations of the salivary bacilli in neutral jelly; and that, after one or two generations, they will grow in the alkaline gelatine; and he says that the comma-bacilli of the mouth are identical with Koch's comma-bacilli in mode of growth "in many respects." In the later paper of April 4th, however, he tells us that, after acclimatisation in neutral media, the salivary bacilli "present the same appearances of growth as the choleraic comma-bacilli." He says also that the cholera-bacilli, under varying conditions, show similar variations, and instances the following: "When the choleraic comma-bacilli are mixed with hydrochloric acid (1 to 1,000), from ten to fifteen minutes, and when, after this, they are sown in alkaline nutritive gelatine, they do not grow at all, or only with great difficulty; but, on sowing them, after the treatment with hydrochloric acid, in alkaline broth, they grow well, and then, transferred to alkaline nutritive gelatine, they show copious and typical growth." This result is, however, not a case of acclimatisation at all, as will be seen by reference to my experiments with acids; had glass-plate instead of test-tube cultivations been made in the first instance, it would have been found that the bacilli grow as readily in the alkaline gelatine as in the meat-infusion, the result in the test-tube cultivations simply depending on the acid being carried along the needle-track along with the bacilli, and impeding or preventing their growth. I may here say that, as the result of his experiments with 1 to 1,000 hydrochloric acid, Dr. Klein states that these bacilli are not killed by acids. This, however, entirely depends on the strength of the acid employed, acid of the strength of the gastric juice killing them, as I have previously shown, very rapidly. It would be interesting to know whether Dr. Klein's dilute acid (1 to 1,000) was one part of the commercial acid to one thousand parts of water, or whether it was made up on Dr. Brunton's formula. If it were the former, then his results entirely correspond with mine; but, in any case, they do not bear in any way on this question of acclimatisation of the salivary bacillus.

Returning now to the salivary bacillus, is it a fact that it will grow in neutral jelly containing five or ten per cent. of gelatine? I have stated in my report that I have failed to cultivate it in this material, and, as I have said, other observers have had like bad success. In fact, I have delayed the publication of this report for several weeks in order to go into this matter thoroughly, and, after numerous experiments, I am satisfied that the salivary comma-bacilli will not grow in this material. Of course, before Dr. Klein's views on the acclimatisation of the salivary bacillus can be accepted, or even discussed, he must give us a method by which anyone with sufficient skill

which will be referred to towards the end of this appendix. His other remarks in the note to which I allude would not have been necessary, had he had patience to wait till my report was finished.

Dr. Klein's other statement about finding cholera-bacilli in ligatured portions of intestine in monkeys, after injections of sulphate of magnesia, is not founded on sufficiently precise facts, and is so improbable, that judgment on this matter must be delayed for the present.

and experience in these matters can repeat his observations. Dr. Koch told us how to cultivate the cholera-bacillus, and his observations can be readily verified by anyone. Dr. Klein has told us how he thinks he has succeeded in cultivating the salivary bacillus, but his experiments cannot be repeated. He has not told us on how many different occasions, and on how many different individuals, he has repeated his observations. Is he perfectly certain that there could not have been accidental contamination of his materials with cholera-bacilli? If Dr. Klein will go to some other laboratory, such as at Oxford or Cambridge, leaving behind him all cholera-cultivations, instruments, etc., so as to avoid the chance of accidental contamination, and if he will there prepare fresh material, and obtain cultivations of salivary bacilli, identical in all respects with Koch's bacilli after acclimatisation in whatever medium he chooses, and will further describe a method by which his results can be repeated, then the matter can be further discussed. But unless that be done, my conviction is that the explanation of his experiments is the same as that which he gave of Buchner's similar experiments with anthrax and hay-bacilli, and the same as the explanation of the old experiments on the artificial production of tuberculosis, namely, accidental contamination.

That I am not making any improbable suggestion is evident from Mr. Dowdeswell's research, published in the BRITISH MEDICAL JOURNAL, March 21st, 1885. Mr. Dowdeswell there tells us that, "in separating some other microbes by fractional cultivation in gelatine," he found that he "had accidentally got a growth of typical comma-bacilli, as far as shown by the characters of the colonies on the surface of the gelatine." Mr. Dowdeswell thinks that the contamination in this case came from the air. Although I think that there are very grave reasons against the view that the contamination in this case came from the air—in fact, I do not feel inclined to accept this explanation without further proof—nevertheless, the observation is extremely interesting and important, as showing that an observer working in the Brown Institution, and with Dr. Klein's methods, is not safe from accidental contamination of his cultivations with comma-bacilli, if cultivations of cholera-bacilli are being carried on at the same time, and in the same place.

I may now pass on to the second statement in the preliminary report, which is the following. "The 'comma-bacilli,' in acute typical cases of cholera, are by no means present in such numbers, and with such frequency, as to justify Koch's statement, that 'the ileum contains almost a pure cultivation of comma-bacilli.'" This statement is reiterated in the several communications to which I have referred. The amount of importance to be attached to this observation entirely depends on the manner in which it was made; and there is no statement that it was an observation as the result of cultivation, but rather as the result of microscopic investigation. The same error of dependence on microscopic characters is evidently at the foundation of this statement as was at the root of the first proposition. I have in my report sufficiently pointed out the fallacy of microscopic observation alone, and I need not go over the ground again. Dr. Gibbes, it is true, referred, at the meeting of the Royal Medical and Chirurgical Society, to cultivations in these cases; but he only referred to test-tube cultivations; there was no mention of estimation by glass-plate cultivations. Dr. Gibbes told us that, when a number of tubes were inoculated from the contents of the intestine in an acute case, the resulting growth, in the majority of instances, contained only a few cholera-bacilli. But, from the results of test-tube cultivations, one cannot gain any idea of the relative numbers of organisms present in the original material. For the organism which finds the nutritive material the most suitable soil for its growth will grow most rapidly, other conditions being favourable, and will very soon be present in

greatest numbers, even though it may not have been the most numerous form in the material originally introduced. In fact, the cholera-bacilli seem very readily to disappear from many mixtures of different bacteria. This observation with regard to the numbers of bacilli in acute cases is entirely opposed to my own experience, as narrated before, and to the experience of others who have worked with the glass-plate method. In any case, it is by no means the most important point, the necessary questions being the constant presence of this bacillus in cholera, and its absence in other instances.

In connection with this matter, I may also refer to Dr. Klein's observation on the tank in which he found "comma-bacilli," although a number of persons were drinking this water without becoming affected with cholera. The question again naturally arises: Was it the cholera-bacillus which was present in this water? Was the microscopic appearance alone trusted to, or was cultivation used as a test? If this statement rests solely on microscopic observation, then we cannot consider the matter further, because we do not know that we have here to do with the cholera-bacillus. The same error vitiates this question as vitiated the former two. But even if we suppose that in this instance it was the cholera-bacillus that was present in the tank, the observation does not prove that these bacilli are not the cause of cholera. As I have pointed out already, many conditions come into play in connection with an attack of cholera. For instance, whatever be the virus of the disease, why does it apparently at times lie more or less quiescent, and then suddenly lead to epidemic outbursts of the disease? Why does one epidemic differ from another in virulence, etc.? But, apart from conditions affecting the virus probably outside the body, there are conditions in the body which may or may not predispose to an attack. Take tuberculosis, for example. There is every reason to believe that the inhalation of tubercle-bacilli will, in man, as in animals, under suitable circumstances, set up a tubercular process. And yet we probably all have inhaled tubercle-bacilli at some time or other without becoming tubercular. Two conditions at least evidently influence this. Thus, for one thing, the tubercle-bacilli may never reach, or be able for mechanical reasons to settle in, a suitable part; or, in the second place, reaching such a part—say, the air-vesicles of the lung—they may not find a suitable soil on which to grow. Or, to take a case about which there can be no dispute—anthrax—we know that, probably according to the state of the individual, one man may escape after inoculation with anthrax-bacilli, another may have a mild pustule, another a severe one, and a fourth an acute fatal disease. So in this case, apart from the degree of virulence of the organism, the bacilli in the water may never have reached the necessary seat—the intestine—or, reaching it, may not have found it a fit soil. For these various reasons, I do not think that much importance can be attached to this observation; and I see that Dr. Klein omits it altogether in his last statement on the "precise position with regard to the comma-bacilli of Koch."

The third statement in the preliminary report is that "the 'comma-bacilli' are not present in the tissues of the intestine or elsewhere." What is meant by "elsewhere" is not clear, but I presume it refers to the blood and various organs. This same statement is reiterated in the various other places to which I have referred. I have previously mentioned the fact that Koch and others have found considerable numbers of these organisms in the tissues of the lower part of the ileum, more especially in the neighbourhood of Peyer's patches, in acute cases in which the *post mortem* examination was made very soon after death. I have also narrated my own failure to find these organisms in the tissue, and given a possible explanation of it. The same explanation is probably applicable to Dr. Klein's results. In any case, it does not seem to me that this matter touches the ques-

tion of the causal relation of these bacilli to Asiatic cholera. Dr. Klein seems to make a very strong point of this, and to think that his failure to find the bacilli in the intestinal wall, is sufficient to fatally affect Dr. Koch's view of the causal relation of these bacilli to Asiatic cholera. It seems to be thought that the cholcraic virus acts by producing a poison, and that, unless the organisms be present in the intestinal wall, any poison formed by them could not be absorbed. Dr. Klein himself said, at the meeting at the Royal Society (BRITISH MEDICAL JOURNAL, February 7th, 1885), that "Koch's theory as to the comma-bacilli, present in the mucous membrane, secreting a chemical poison inducing the disease, cannot, therefore, be correct." Here two views are confused together; in fact, a third view is being tacked on, to which Dr. Koch never gave expression. Dr. Koch says that the cholera-bacilli are the cause of cholera, because they are always present in the contents of the intestine, and are never found in other circumstances than in association with Asiatic cholera. Then he says, accepting the view that they are the cause of the disease, I think that they probably act by producing a poison which is absorbed, and which gives rise to the symptoms. And then the view is tacked on to this, that it is while growing in the wall of the intestine that they produce this poison, a view to which Dr. Koch never gave utterance. All that is essential for Koch's view that the cholera-bacilli are the cause of cholera is their constant presence; and it does not matter for this view whether the cholera-bacilli are present in the walls of the intestine or only in its contents, so long as they are constantly present somewhere or other in the affected part. The idea that they act by producing a poison which is absorbed, and gives rise to the symptoms, though probable, is a mere working hypothesis, and may have to be modified according as fresh facts are made out, without in any way invalidating the main position that the cholera-bacilli are causally related to cholera. Nor, even if it were necessary for this view that a poison should be formed, does it follow that it could only be absorbed if formed in the wall of the intestine, and not if formed in the intestinal canal.

In the BRITISH MEDICAL JOURNAL for April 4th, 1885, Dr. Klein states that, in several typical rapidly fatal cases, the whole of the small intestine "presented an uniform appearance; the alterations extended equally to the whole small intestine. But in these cases there were no comma-bacilli present except in the lower part of the ileum, and here they were present in very small numbers indeed, the *post mortem* examination having been made very soon after death." This statement is entirely opposed to the results which I obtained in case No. 8, in which I examined the contents of the middle part of the jejunum as well as of the lower part of the ileum, and did not find any marked difference in the number of cholera-bacilli present in the two places. Nor does Dr. Koch, in his reports, state that cholera-bacilli are only present in the contents of the intestine at the lower part of the ileum, though he has found them in the mucous membrane only in that situation. The same error of dependence on microscopic appearances alone renders it impossible for us to accept Dr. Klein's observation.

We now come to the fourth statement in the preliminary report. "The 'comma-bacilli' in artificial cultivations carried out by one of us (E. K.), do not behave in any way differently from other putrefactive organisms." The exact meaning of this statement was for a long time quite unintelligible to me. There are, I think, at least two distinct views implied here; 1, that the cholera-bacillus is a putrefactive organism—the word "other" implies that; and 2, that there is some common mode of behaviour of putrefactive organisms in artificial cultivations. As regards the first point, it is, of course, very difficult to define what is meant by a putrefactive organism, but I

should define it as one that produces or aids in producing putrefaction. In this sense I deny that the cholera-bacillus is a putrefactive organism; it produces at most a slightly fæcal odour, and is never found in putrefying materials. In connection with the second view, Dr. Klein says, in a letter to the BRITISH MEDICAL JOURNAL, on January 24th, 1885, "that anyone sufficiently familiar with cultivations of the various species of putrefactive bacteria in solid media, knows that almost every species—and even one and the same species cultivated in different media—exhibits peculiarities of its own, which in many instances are so marked, that an expert is able to distinguish them even with the unaided eye." There is no mention here of any character in cultivation peculiar to all putrefactive organisms which is also possessed by the comma-bacillus; on the contrary, he says, "anybody who has the opportunity and skill to make pure cultivations of these" (the cholera) "organisms in gelatine, cannot fail to find that Koch's description of the peculiar behaviour of the comma-bacilli under these conditions is, in all its details, absolutely correct." The first light which I got on this matter was from the sentence which followed the above; "but to conclude from this peculiar behaviour of Koch's comma-bacilli in gelatine, and from the manifest differences existing in this respect between them and Finkler's comma-bacilli, that the comma-bacilli of Koch are not putrefactive, but specific organisms, is a proposition which is as unsound in logic as it is incorrect in fact." At the meeting at the Royal Medical and Chirurgical Society, Dr. Klein made his meaning quite clear, by telling us that Dr. Koch considered that these organisms were pathogenic because, among other reasons, they grew in a particular manner on nutrient jelly. Indeed, at the Royal Society the same view had been stated (see BRITISH MEDICAL JOURNAL, February 7th, 1885). There Dr. Klein says, "on account of their constant occurrence in the intestines of patients suffering from Asiatic cholera, on account of their absence in all other diseases of the intestine, and *on account of their peculiar mode of growth in nourishing gelatine*, Koch claims for these comma-bacilli not only an important diagnostic value, but also considers them as the true cause of cholera." How such an idea could have arisen it is difficult to imagine, for Dr. Koch never said, nor even supposed, that they were pathogenic because they grew in a special manner on nutrient jelly. I am not aware that in the case of any organism, however virulent, it would be possible to say, from the appearance of the cultivation, that the organism would prove, on trial, to be or not to be pathogenic. Certainly such an idea has never been entertained by Dr. Koch, nor, so far as I know, by any of his followers. Dr. Koch lays stress on the cultivation-characters of this organism as furnishing a means of distinguishing it from other organisms, and not as affording an argument in favour of its pathogenic nature. I pointed this out at the meeting at the Royal Medical and Chirurgical Society, and I see that Dr. Klein does not bring this forward as an argument against Dr. Koch, in either of the two papers which he has published since.

The two errors, then, which, in my opinion, lie at the root of the work of the English Commission are, first, that, acting on the idea that Koch diagnosed the cholera-bacilli by the microscope alone, they proceeded to investigate the matter by microscopic examination; and, secondly, that, seeing the stress which Dr. Koch laid on the cultivation-appearances, they concluded that he meant to say that the organism was pathogenic, because it grows in a particular manner; and, therefore, they naturally proceeded to inquire whether the appearance of the cultivations, as compared with cultivations of other bacteria, could warrant this conclusion, and, of course, they found that it could not.

I need not go into detail on the other matters in Dr. Klein's re-

search, because they are of very secondary importance; but it may be well if I briefly refer to two points. The fifth statement in the preliminary report is this: "Mucus-flakes of the ileum, taken out soon after death from typical acute cholera, contain numerous mucus-corpuscles, many of them filled with peculiar minute straight bacilli. The same bacilli occur also outside the mucus-corpuscles. They are never missed, even where the 'comma-bacilli' are." After thinking over this matter in connection with my own experience, and after seeing Dr. Klein's specimens at the meeting at the Royal Medical and Chirurgical Society, I ventured to suggest at that meeting that it was possible that these bacilli are only young cholera-bacilli, and I thought so for two reasons. In the first place, it is precisely in these mucus-flakes that one finds, on cultivation, that the largest numbers of the cholera-bacilli are present; while, on microscopic examination, there may, as Dr. Klein says, appear to be very few of these organisms: hence some of the forms in which the curve is not very marked must also be cholera-bacilli. And, in the second place, the specimen which I saw at the meeting looked very like a specimen of young cholera-bacilli (see Fig. 1). Of course, I know that the reply to this is, that Dr. Klein has cultivated these bacilli, and has found that they are not cholera-bacilli. My answer to that is that, without doubt, Dr. Klein has cultivated small straight bacilli from the contents of the intestine, but that is no proof that the bacilli cultivated were derived from the bacilli seen in the mucus-corpuscles; it is impossible to make a cultivation from a single mucus-corpuscle, without getting at the same time the other bacteria which are present in the contents of the intestine. Small straight bacilli, similar in growth to those exhibited by Dr. Klein, can be cultivated from the contents of normal intestines, and would naturally be present in the contents of a choleraic intestine. After all, it seems to me that these straight bacilli do not deserve the attention they have received; for Dr. Klein has never said that they only occur in the intestine in Asiatic cholera; and, unless that can be demonstrated, they evidently have no real connection with the disease.

Dr. Klein also attributes the results in the successful cases of inoculation of cholera-bacilli to septicæmia, or to the effects of the operation. The former idea is incorrect, because the operations, both in Dr. Koch's cases and in mine, were performed strictly antiseptically; and in the guinea-pigs, in my case, there were no bacteria in the blood and tissues. Dr. Klein refers, as a similar example, to fatal epidemics of diarrhœa in rabbits, in which there are no bacteria in the blood or internal organs. Of this I am quite aware, and it was to an epidemic of this kind that I referred in Series I and II in my report on micrococci in relation to wounds, etc. (BRITISH MEDICAL JOURNAL, September 20th, 1884). But the animals on which I experimented with cholera-bacilli in the way described were guinea-pigs, and they are not, so far as I know, subject to similar epidemics. I have never operated on rabbits, because I understood that they were quite refractory to the cholera-bacillus.

From what has been said, it will be evident that no facts have been brought forward in opposition to Dr. Koch's research which can bear criticism. Dr. Koch's facts remain now as correct as when he first published them. He said that this organism was constantly present in Asiatic cholera, and that it was present nowhere else. These statements have now been amply confirmed; and it is perhaps the most striking evidence in favour of the precision of Dr. Koch's methods, and of the care which he has devoted to the subject, that, though other bacilli, of somewhat similar appearance, have since been discovered, yet, by the use of his methods, it has been found easy to distinguish them one from another, and from the cholera-organism.

Appendix B.

The following is the letter referred to on page 38 :—

THE CHOLERA-BACILLUS OF KOCH.

Sir,—In the Journal for last week, Dr. Klein makes "some remarks on the present state of our knowledge of the comma-bacilli of Koch," which he divides into two parts, (1) reference to the part of my speech at the Royal Medical and Chirurgical Society, in which I referred to Koch's method of investigating the cholera-bacillus ; and (2) his own views on the subject of the cholera-bacillus. The second part of these remarks deserves careful consideration, and I shall take the opportunity of referring to them in a paper which I hope to publish in the Journal very shortly, The first part raises, however, a purely side-issue, of little consequence at the present time, and hardly worthy of consideration in a scientific paper. I, therefore, think it best to take the present opportunity of dealing with it.

I maintained that Koch's definition of cholera-bacilli was not merely that they were comma-shaped, but that they possessed other characteristics, more especially of growth, by which they could be distinguished from other bacteria ; that, in fact, his definition was the sum of all their characters. I further stated in this connection that Dr. Koch had searched for these organisms in saliva, fæces, etc., without finding them ; and that, when Dr. Koch said that he had searched for these organisms, he meant that he had not only used the microscope, but had tested the materials by cultivation ; and when Dr. Koch said that he had failed in this search, he meant that he had not found an organism which presented the same microscopic appearance, together with the same characteristics of growth, as that discovered by him in Asiatic cholera. Dr. Klein says : "This is what Mr. Watson Cheyne says, but it is not what Koch said." But Dr. Klein is, as I shall now show, quite mistaken when he makes this statement. Dr. Klein falls into the error, to which I was referring when I spoke, of supposing that Dr. Koch in his research diagnosed cholera-bacilli by their microscopic appearance alone, a mistake which vitiates the whole investigation of the English Commission.

Dr. Klein says : "Where has Koch stated that the comma-bacillus is peculiar to cholera, and must, therefore, bear a definite relation to the disease ? In Egypt. All the beautiful investigations as to the peculiar behaviour of the choleraic comma-bacilli in gelatine were made by him subsequently in Calcutta. But their discovery, their description, and their specific relation to cholera, were asserted by microscopic examination only." The statement in the last sentence is incorrect, as will be evident from the quotations which I shall now make. If we turn to Koch's first report (*Fortschritte der Medicin*, November 1st, 1813, Beilage, p. 183), dated Alexandria, September 17th, 1883, we find the following statements with reference to the presence of

comma-shaped bacilli in the intestinal mucous membrane. "As, however, the bacilli were found in all fresh cholera-corpses, and, on the other hand, were absent in the cases examined after the attack had passed off, as well as in several other cases which had died of different diseases, and were likewise investigated for the sake of comparison, there can be no doubt that they stand in some relation to the choleraic process. Nevertheless, one cannot conclude, from the fact of the presence of the bacilli in the intestinal mucous membrane in cases of cholera, that the bacilli are the cause of that disease. It might, on the contrary, be that the cholera process sets up such changes in the intestinal mucous membrane, that, of the numerous putrefactive bacteria constantly present in the intestine, the penetration into the tissue of a particular form was rendered possible." In the fifth report, dated January 7th, 1884, from Calcutta (*Fortschritte der Medicin*, March 1st, 1884, Beilage, p. 33), we have the next important reference to these bacilli: "In my report of September 17th, I had to leave it undecided whether these bacilli did not, like so many other bacteria, belong to the ordinary parasites of the human intestine, and only penetrated into the intestinal mucous membrane under the influence of the morbid changes of cholera. *Sufficient characteristics were at that time wanting to enable us to distinguish these bacilli from other very similar forms of intestinal bacilli.*[1] This want has now been fortunately remedied. For, with the help of the methods perfected in the Sanitary Institute, which have also, in this instance, answered admirably, we succeeded in isolating the bacilli from the intestinal contents in the most typical cases of cholera, and in carrying on pure cultivations. The careful observation of the bacilli in their pure cultivations led then to the discovery of several very characteristic peculiarities with regard to their form and mode of growth in nutrient gelatine, whereby they could be distinguished with certainty from other bacilli. Thereby the means were obtained of definitely deciding the question whether these bacilli belonged to the ordinary inhabitants of the intestines, or whether they were exclusively present in cases of cholera." In presence of this fact, what becomes of the statement made by Dr. Klein that "their description and their specific relation to cholera were asserted by microscopic examination only?"

In the second place, Dr. Klein objects to my statement that, in searching for these organisms in fæces, saliva, etc., Dr. Koch did not rely on their microscopic appearance alone, but also on their characters on cultivation; and he tries to support his opinion that Koch only used the microscope for diagnosis, by various extracts from Dr. Koch's report. I shall, in the first place, continue to quote from the preliminary reports. In the fifth report, referred to above, Dr. Koch says: "At first, the bacilli were demonstrated in the dejecta of cholera-patients, and in the intestinal contents of fatal cases, *by the aid of the gelatine-cultivations*, and this was successful in all the cases investigated here, Then, also, the intestinal contents of other dead bodies were examined *in like manner*, and it was found that the bacilli of the cholera-intestine were always absent." In the sixth report (*Fortschritte der Medicin*, April 1st, 1884, Beilage, p. 49), he enters into details with regard to these diagnostic characters of the cholera-bacilli, and then goes on to say, "Up to the present time (February 2nd, 1884), in Calcutta, 22 dead bodies and 17 cholera-patients have been examined. *All these cases have been tested by gelatine-cultivations, as well as by microscopic examination*, etc." "For control, the following were examined *in exactly the same manner;*" and then follows a list of the materials examined. I ask the unprejudiced reader if I was not, therefore, correct when I stated that, when Koch said that he had searched for these organisms he meant

[1] The italics throughout are mine.

that he had not only used the microscope, but had tested the materials by cultivation. But, as Dr. Klein has been quoting from Dr. Koch's full report, I may show that there also the same fact is distinctly stated. Dr. Klein quotes the following sentence: "As a test, a considerable number of other corpses, dejecta from patients and persons in good health and other substances containing bacteria, were examined *in the same manner*, to see if these bacilli, which were never missing in cases of cholera, might perhaps occur elsewhere also." Dr. Klein omits a very important expression in this sentence, namely, "in the same manner," and merely mentions in a footnote that it occurs, and that it refers "to a previous paragraph, in which he (Dr. Koch) states that he had examined microscopically nearly 100 cases in all for the presence of comma-bacilli, and had found them always." The whole question is in what manner Koch investigated this material, and when Dr. Klein states that the phrase refers to cases examined microscopically, he makes a mistake, as will be immediately evident. Let us consider this paragraph to which the phrase "in the same manner" applies. It begins on page 21 of Koch's report, but perhaps I may quote the previous paragraph as confirming the quotations which I have been making from the preliminary reports.

"If one takes into consideration all the characteristics of the comma-bacilli which have now been described, one must be convinced that they belong to a distinct and well characterised species of bacteria, and that they may be readily recognised and distinguished from other bacteria *by the help of their peculiar characteristics.*"

Now comes the paragraph to which Dr. Klein refers. "After this conviction was obtained, it was necessary, before all things, to establish the relation which the comma-bacilli bear to the choleraic process, and in the first place it was necessary to ascertain whether they were present in all cases of cholera, and whether they were absent in non-choleraic cases; in other words, whether they belong exclusively to cholera. In this direction as large a number of cases as possible was very thoroughly examined. In Egypt use could be made of ten *post-mortem* examinations; these were, however, only microscopically investigated, etc." (observe how careful Koch is to indicate when the microscope alone was employed). "In India, 42 fatal cases were examined, *both microscopically, and also by cultivation in nutrient gelatine*, and the bacilli were never absent." ..... "Further, in India the dejecta of 32 cholera-patients were examined *in like manner*," etc. The manner, then, in which he investigated these cases, was to make microscopic examination of sections of solid tissues, and to test other materials by cultivation as well as by the microscope. And, therefore, when he states that he has examined a large number of other substances for comma-bacilli "in the same manner," it can only mean that he examined sections with the microscope, but that other materials were tested by cultivation as well. And yet Dr. Klein says that "there is nowhere a word of a culture-test."

Dr. Klein further says: "I challenge anyone to show me in Koch's pamphlet..... one single word that would indicate that he had the slightest idea that there occur comma-bacilli other than those in cholera." If Dr. Klein will turn to page 25 of Koch's report, he will find the following answer to this challenge. In speaking of his researches with various fluids containing bacteria, Koch says, "Only once did I find in the water, which at the time of the floods overflowed the region of the salt-water lake lying eastward from Calcutta, a form of bacteria which at the first glance, had a certain likeness to the cholera-bacilli, but by accurate examination they appeared somewhat larger and thicker, and their cultivation did not liquefy the gelatine." Does he not here plainly say that he found a comma-shaped bacillus, which, however, was somewhat larger and thicker than the cholera one, and which did not liquefy gelatine? I have already quoted Dr.

Koch's statement, in his earlier reports, that he had to search for other characteristics in order to distinguish these bacilli from "others very similar forms of intestinal bacilli;" and in my speech I referred to the bacillus of glanders which is often curved in the tissues, and to which, on account of resemblance in size and form in the tissues, Koch compared the cholera-bacillus. That Dr. Koch did not at that time know of the existence of Finkler's and Flügge's comma-bacilli is certain, but it is equally certain that from the very first he felt the necessity for additional characteristics to those furnished by the microscope in order to enable him "to distinguish these (the cholera) bacilli from other very similar forms of intestinal bacilli." I need not pursue this subject further, although I could produce other passages from Dr. Koch's reports in justification of my assertions, which it will now be evident were strictly accurate.

So long as Koch has demonstrated a distinct species of organism in cholera, it would not much matter how long he had taken to find out the characters necessary to diagnose it, were it not that the supposition that Koch diagnosed it by its form alone was one of the chief sources of the errors in the preliminary reports of the English Commission. For, acting on this belief, they naturally proceeded to examine the evacuations of dysentery, diarrhœa, etc., by the microscope for comma-shaped bacilli; and, finding them, made a great point of their presence as proof against Koch's views, quite omitting to state whether or not they were the same organisms as the cholera-bacilli. Now, however, it turns out that they were not the same organisms, and thus the belief engendered in the minds of many who reads these reports, that Koch was wrong, because the same bacilli were present in dysentery, etc., as in cholera, falls to the ground. But with this, and the other points in Dr. Klein's researches, I shall deal in another communication.—I am, sir, yours faithfully.

W. Watson Cheyne.